CHEMICAL REGULATION OF THE GROWTH AND REPRODUCTION OF SOME BLUE GREEN & GREEN ALGAE

Thesis Submitted to the University of Allahabad

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

SCIENCE

By

UMA MISRA

M.Sc.

DEPARTMENT OF BOTANY

UNIVERSITY OF ALLAHABAD

ALLAHABAD (U.P.) 211002

2002

DEDICATED

TO

MY PARENTS

Department of Botany
University of Allahabad
Allahabad-211002
Phone : 461887

Dated 23.4.2002

CERTIFICATE

I have pleasure in forwarding the thesis of Mrs. Uma Misra, M.Sc. entitled "Chemical regulation of the growth and reproduction of some blue-green and green algae" for acceptance for the degree of Doctor of Philosophy in Botany.

Mrs. Uma Misra has worked in this laboratory from 12.8.1999 to 30.10.2001.

Dated :

(S.C. Agrawal)
Supervisor

ACKNOWLEDGEMENTS

I express my deep sense of gratitude to Dr. S.C. Agrawal, Reader, Department of Botany, University of Allahabad, Allahabad for his suggestions, encouragement, inspiration, guidance and constructive criticism which enabled me to complete this work.

I wish to express my sincere thanks to Prof. G.L. Tiwari, ex Head, Deptt. of Botany, University of Allahabad, Allahabad for his help and suggestion during my research work.

I am deeply grateful to Prof. D.R. Mishra, Head, Deptt. of Botany, University of Allahabad, Allahabad for all the research facilities provided.

I am also thankful to Dr. (Mrs.) G. Dixit, Dr. (Mrs.) Madhu Sengar, Dr. (Mrs.) Vandana Singh, Dr. Kirti Raje Singh, Ms Jyoti Jaiswal, Mrs Uma Pal, Ms Richa Tandon, Ms Suman Gupta, Mr R.K. Ojha, Mr S. Upadhya, Mr Y.N. Pandey and Ajay for their help and cooperation during my research work.

I express my deep regards to my parents Sri H.P. Misra, Rtd. I.P.S., Mrs. Deval Misra for inspiring to start my work. I am thankful to Sri G.S. Pandey, Mrs L.M. Pandey, Sri Rajesh Misra, Mrs Aparna Misra and all family members for cooperation in completing the thesis.

I gratefully acknowledge help of my husband Er. Prabhaker Pandey and children Kartika and Shantanu for their suggestions and generous cooperation in completing my research work.

Uma Misra

Date : 23.04.02

(Uma Misra)
Department of Botany
Universit of Allahabad
Allahabad

CONTENTS

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Very little information is available regarding chemical regulation of growth and reproduction in Algae. Laboratory culture studies indicate that algal growth, reproduction and germination of resistant cells, all are controlled by a variety of inorganic and organic chemicals (Reviewed by Coleman, 1962; Erben, 1962; Dring, 1974; Agrawal, 1998).

Ernst (1908) observed that nitrate or phosphate starvation induced akinete formation in *Pithophora oedogonia*. Anderson (1960) obtained good growth of *Chara* sp. without addition of phosphate to the culture medium. Lack or limitation of phosphorus has also been found to increase akinete formation in *Anabaena* spp. (Wolk, 1965; Kaushik *et al.*, 1971; Van dok and Hart, 1996), *Cylindrospermum* sp. (Wolk, 1965; Kaushik *et al.*, 1971), *Aphanizomenon flos-aquae* (Gentile & Maloney, 1969) and *Nodularia spumigena* (Pandey & Talpasayi, 1980).

Nitrogen deficiency has been found to induce reproduction in some algae like *Oedogonium* sp. (Hall, 1980), *Chlorococcum* sp. (O'Kelley, 1984), *Gloeotrichia* sp. (Wyman & Fay, 1986), *Chlamydomonas* sp. (Van den Ende *et al.*, 1992) and in *Peridinium* spp. (Chapman & Pfiester, 1995).

Presence of calcium and magnesium promoted flagella agglutination in *Chlamydomonas* (Wiese & Jones, 1963; Tomson *et al.*, 1986) and conjugation in *Mesotaenium* (Tiftickjian & Rayburn, 1986).

Kretschmer (1930) first showed that pH or hydrogen ion concentration of the culture medium on alkaline side was suitable for the formation of zoospores and sex organs in *Oedogonium* sp. The formation of cysts in *Platymonas* sp. (Tanoue & Aruga, 1975) and that of the spores in *Eunotia* sp. (Von Stosch & Fecher, 1979) was enhanced within very short period of time either by raising the pH of cultures to 9.0 or by lowering it to 6.0.

Moore (1967) observed that pesticide Vapam did not produce any change in growth of *Nostoc muscorum* at 1 ppm. Some strains of *Anabaena* and *Nostoc* were found to grow well even at 100 ppm of pesticide Delapon (Venkataraman and Rajyalakshmi, 1972). BHC powder at 10 ppm stimulated the growth of *Aulosira fertilissima* (Ahmed & Venkataraman, 1973), while Cypermethrin and Fenvalerate at 5 ppm could stimulate the growth of blue-green algae in rice fields (Megharaj *et al.*, 1986).

Application of carbofuran at 0.5 to 1 kg/ha levels to soil slightly enhanced the growth of blue-green algal population (Megharaj *et al.*, 1988).

Pesticides Furadan, Sevin and Rogor at about 10 ppm could increase the growth of *Westiellopsis prolifica* (Adhikary, 1989). *Aulosira fertilissima* and *Nostoc muscorum* grew maximally with 50 ppm of pesticides Malathion, Phosphomidon or Dichlorovos in abscence of inorganic phosphate in the medium (Subramanian *et al.*, 1994).

Some heavy metals are essential for growth and other metabolic processes of algae. Manganese was required for growth of some algae

(Rai *et al.*, 1981a), while Nickel stimulated growth in *Chlorella vulgaris* (Stokes, 1975) as well *Nostoc muscorum* (Rai & Raizada, 1986). Cobalt in the form of Vitamin B_{12} was required to increase the dry weight of marine algae *Ulva, Dictyota* and *Pterocladia* (Nasr and Bakheet, 1970).

Indole acetic acid at low concentrations increased the formation of zoospores in *Ulothrix* (Conrad *et al.*, 1959), spores in *Derbesia* (Hustede, 1964), aplanospores in *Trebouxia* (Giles, 1970), akinetes in *Stigeoclonium* (Agrawal & Sarma, 1984), caps in *Acetabularia* (Driessche, 1984) and Oogonia in *Oedogonium* (Singh & Chaudhary, 1988).

Gibberellic acid at low concentrations increased the formation of zoospores in *Ulothrix* (Conrad *et al.*, 1959), akinetes in Stigeoclonium (Agrawal & Sarma, 1984) and oogonia in *Oedogonium* (Singh & Chaudhary, 1988). Kinetin at low concentrations stimulated the cap formation in *Acetabularia* (Spencer, 1968) and sexuality in *Chlamydomonas* in dark (Ishiura, 1976).

Abscisic acid at low concentrations induced sexuality in *Chlamydomonas* in dark (Ishiura, 1976).

The aim of the present study was to find out that in what way The following

(i) amendments in nutrients concentration of the culture medium,

(ii) pH of the culture medium,

(iii) presence of some pesticide, like carbofuran, phorate, 2,4-D, dithane or bavistin in the culture medium.

(iv) presence of some heavy metals like copper sulphate, Zinc oxide, mercuric chloride, lead nitrate or Cobalt nitrate in the culture media, or

(v) that of growth hormones such as 3-Indole acetic acid, Gibberellic acid and Kinetin in the culture medium did effect the survivability of vegetative cells, akinete formation and germination in blue-green algae *Anabaena iyengarii* var tenuis RAO, *Westiellopsis prolifica* JANET, *Nostochopsis lobatus* WOOD; the survivability of vegetative cells and akinete formation and germination in green-alga *Pithophora oedogonia* (MONT) WITTROCK; and the survivability of vegetative cells and zoosporangium formation and zoospore germination in green-algae *Cladophora glomerata* (L) KUETZING and *Rhizoclonium hieroglyphicum* (C.A.Ag) KUETZING.

These algae were chosen for the present study because most of them were easily available from the local habitats. They also grow well in cultures and forms reproductive structures like akinetes or zoosporangia. Akinetes or zoospores of the present algae germinates prolifically without any dormancy.

This study will be helpful to know that whether chemical stresses like that of lack or abundance of nutrients, pH extremes, presence of some pesticides or heavy metals in the culture medium could induce reproduction in the present algae or not and also that whether growth hormones could induce reproduction or spore germination in algae.

CHAPTER II

EXPERIMENTAL ALGAE AND CULTURE MEDIUM

CHAPTER II

EXPERIMENTAL ALGAE AND CULTURE MEDIUM

EXPERIMENTAL ALGAE

Blue-green and green algae used in the present study were

(i) ***Anabaena iyengarii*** **var.** ***tenuis*** **RAO**

(ii) ***Westiellopsis prolifica*** **JANET**

(iii) ***Nostochopsis lobatus*** **WOOD**

(iv) ***Pithophora oedogonia*** **(MONT.) WITTROCK**

(v) ***Cladophora glomerata*** **(L.) KUETZING, and**

(vi) ***Rhizoclonium hieroglyphicum*** **(C.A.Ag.) KUETZING**

Anabaena iyengarii var. *tenuis* RAO

SITE OF COLLECTION

Alga *A. iyengarii* was collected from a fresh water pond at Allahabad.

MORPHOLOGY OF VEGETATIVE CELLS, HETEROCYSTS AND AKINETES

The trichomes of the alga were either straight or irregularly curved. They had vegetative cells measuring about 3.0 to 4.0 µm broad and 3.0 to 4.0 µm long. The end cells of the trichomes were conical with rounded apex. The heterocysts were barrel-shaped, rarely spherical, measuring about 3.0 to 4.5 µm broad and 4.5 to 5 µm long. About 2% vegetative cells differentiated into heterocysts within 15 days of inoculation in the medium.

The akinete formation started in 30 days old culture and the percentage of akinete formation increased progressively upto 90 within 60 days of inoculation. The akinetes were ellipsoidal, measuring about 4.5 to 7.5 µm broad and 9.0 to 13.5 µm long. They were formed in short or long chain on both side of heterocyst (Plate 1).

THE GERMINATION OF AKINETES

Mature akinetes of *A. iyengarii* harvested from old culture medium germinated directly into new trichomes when transferred to fresh culture medium. Akinete germination was initiated 5 days after inoculation and increased upto about 35-50% within 10 to 15 days of inoculation. Akinete germination was initiated with a change in colour

from yellowish-green to dark green and the formation of a small protuberance erupting the cell wall (Plate 1). The clonal cultures of the alga were maintained through germinating akinetes. The alga resembles with that of Desikachary (1959).

Westiellopsis prolifica JANET

SITE OF COLLECTION

Alga *W. prolifica* was collected from a paddy field at Allahabad.

MORPHOLOGY OF TRICHOMES, VEGETATIVE CELLS, HETEROCYSTS AND AKINETES

W. prolifica is a branched filamentous blue-green alga. The vegetative cells of the main axis of the alga were constricted at cross walls and measured 5.0 to 8.0 µm broad and 4.0 to 7.0 µm long, while those of the branches were straight, without any constriction and measured 2.0-3.0 µm broad and 3.0-6.0 µm long (Plate 2).

About 2% of vegetative cells of the alga differentiated into heterocyst within 15 days of inoculation in the culture medium. The percentage of heterocysts did not change much thereafter. The heterocysts of the alga were oblong cylindrical and measured 4.5 to 6.0 µm broad and 8.0 µm to 10 µm long.

The cells of the main axis differentiated into akinetes prior to those of branches. The akinete formation was initiated with a change in colour of vegetative cells from blue-green to dark blue-green and an increase in cells breadth. Each vegetative cell of the alga differentiate into an akinete.

The mature akinetes of the alga were spherical, yellowish-green in colour and measured 6.0-10 µm in diameter (Plate 2).

GERMINATION OF AKINETES

The germination of akinetes was initiated 6 days after inoculation in fresh culture medium and reached to about 30-55% within 10-15 days of inoculation (Plate 2).

The germination of akinetes was initiated with changes in colour of akinetes from yellowish-green to blue-green and division of their protoplasts into 4 germ cells. The germination was completed by an emergence of a protuberence erupting the cell wall.

The clonal cultures of the alga were maintained through germinating akinetes. The alga resembles with that of Desikachary (1959).

Nostochopsis lobatus WOOD

THE SITE OF COLLECTION

The algal material was collected from a paddy field at Pratapgarh.

THE MORPHOLOGY OF ALGA

Nostochopsis lobatus existed in the form of lobed thallus. The trichomes of the alga were richly branched and the branches were scattered. They had barrel shaped vegetative cells measuring 4.0 to 5.0 μm broad and 10 μm long.

The heterocysts were intercalary, lateral as well as terminal, but lateral and terminal heterocysts did not develop much in culture. About 2% of vegetative cells of the alga differentiated into heterocysts within 15 days of inoculation in liquid BG 11 (-N) medium. The percentage of heterocyst did not change much thereafter. The intercalary heterocysts were ellipsoidal. They measured 3.0 to 5.0 μm broad and 4.0 to 6.0 μm long.

The vegetative cells of the alga differentiated into akinetes by changing their colour from blue-green to dark blue-green and then to yellowish-green and by a slight increase in the size. Mature akinetes were spherical, 6.0 to 7.5 μm in diameter (Plate 3).

The akinete formation was not a synchronous process and it increased progressively from 50 to 90% within 30 to 60 days of inoculation.

GERMINATION OF AKINETES

Mature akinetes harvested from old culture medium when transferred to fresh culture medium germinated directly into new trichomes. The germination was initiated 6-7 days after inoculation and reached to about 30 to 60% on 15 days of inoculation (Plate 3).

The clonal cultures of the alga were raised through germinating akinetes. The alga was identified with the help of Desikachary (1959).

Pithophora oedogonia (MONT.) WITTROCK.

SITE OF COLLECTION

The algal material was collected while growing attached to walls of a fresh water pond in my department.

MORPHOLOGY OF FILAMENTS, VEGETATIVE CELLS AND AKINETES

The filaments of *P. oedogonia* were slender, branching solitary or opposite. The vegetative cells were long, cylindrical, about 45 to 70 μm in diameter and up to 696-885 μm in length. *P. oedogonia* reproduced through the formation of akinetes. The akinetes were formed by the contraction of the greater part of the protoplasm of cells towards one end (usually the upper end), separation of that end by a septum and a subsequent development of a thick wall upon maturation (Plate 4).

The akinetes were cylindrical when intercalary and conical when terminal. They measured 59.0 to 88.0 μm in diameter and 147 to 224 μm in length.

THE GERMINATION OF AKINETES

Akinetes harvested from old culture medium germinated directly into new filaments when transferred to fresh culture medium (Plate 4).

The clonal cultures of the alga were raised through germinating akinetes. The alga was identified with the help of Prescott (1962).

Cladophora glomerata (L.) KUETZING

SITE OF COLLECTION

The algal material was collected while growing attached to cement walls of a shallow water tank of my department.

MORPHOLOGY OF FILAMENTS, VEGETATIVE CELLS, ZOOSPORANGIA AND ZOOSPORES

The filaments of the alga were profusely branched and were attached to walls of tank by means of rhizoids. The branching of the alga was lateral and appears dichotomous or trichotomous in arrangement. The branches arise as lateral out growth near the upper end of cells just below the septum. The cells of the main axis of the filaments were 59.0 to 78.0 μm in diameter and were 6 to 7 times of diameter in length (354 μm to 560 μm). The cells in branches were 17.0 to 21 μm in diameter and 118 to 236 μm in length. About 8% of vegetative cells of the alga differentiated into zoosporangia on 15 days of inoculation and the percentage of zoosporangia increased to about 20 after 45 days of inoculation.

An entire vegetative cell of the alga differentiated into a zoosporangium. The zoosporangium formation started from the apical end of the filament and proceeds towards the lower end.

The zoosporangium had a dark green content and was upto 1.5 times broader than vegetative cells. The zoospores were formed in large number and move fast inside the zoosporangium just before escaping through a pore one by one in the medium (Plate 5).

The zoospores were small and biflagellate. They swam in the medium for few minutes and then settled to the walls and to the bottom of the flask. They started germinating within 10 days of attachment. The percentage germination increased to about 65 within 15 days of inoculation.

The clonal cultures of the alga were raised through germinating zoospores. The alga was identified with the help of Prescott (1962).

Rhizoclonium hieroglyphicum (C.A. Ag.) KUETZING

THE SITE OF COLLECTION

The algal material was collected from a running water shallow tank in the university campus. It was growing firmly attached to cement wall of the tank.

THE MORPHOLOGY OF FILAMENTS, VEGETATIVE CELLS, ZOOSPORANGIA AND ZOOSPORES

The filaments of *Rhizoclonium* were unbranched. The cells were cylindrical, much longer than broad. They measured 30.0 to 42.0 μm in diameter and 182 to 253 μm in length. They have thick laminated cells wall.

About 14% of vegetative cells of the alga differentiated into zoosporangia on 15 days of inoculation and the percentage of zoosporangium formation increased up to 35 after 45 days of inoculation.

The zoosporangium had a dark green content and was upto about 1.5 times broader than vegetative cells. It was 50.0 to 53.0 μm in diameter and 107 to 118 μm in length. The zoospores were formed in large number. They move fast inside the zoosporangium just before release into the medium through a lateral pore one by one (Plate 6).

The released zoospores swam in the medium for few minutes and then settled down to the cell wall of the parent filaments. The zoospores started germination after 10 days of attachment. The percentage of zoospore germination increased from about 25 to 55 within 15 days of inoculation.

THE CULTURE MEDIUM AND CULTURE METHODS

In the present work all algae were grown in BG-11 culture medium (Stanier *et al.*, 1971). It is the modified form of G-11 medium (Hughes *et al.*, 1958).

The Chemical Composition of BG-11 Medium

BG-11 medium has following chemical composition :

Name of compound	Gm/litre
$NaNO_3$	1.5
$MgSO_4.7H_2O$	0.075
K_2HPO_4	0.04
$CaCl_2.2H_2O$	0.036
Na_2CO_3	0.020
Ferric Ammonium Citrate	0.006
Citric Acid	0.006
EDTA (disodium magnesium salt)	0.001
Trace metal mix	1 ml/litre

The constituents of trace metal mix were as follows :

Trace Metal mix contents	Gm/Litre
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$Na_2MoO_4.2H_2O$	0.39
$ZnSO_4.2H_2O$	0.222
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.H_2O$	0.0494

The pH of the medium was adjusted at 7.5 prior to autoclaving either by adding 1N HCl or 2% NaOH solution.

The culture medium and glasswares were sterilized in an autoclave at the temperature of 121°C and pressure of 15 $lb/inch^2$ for 30 minutes. In order to avoid precipitation phosphate was sterilized separately and was added to the medium under sterile conditions.

In all cases, inoculation was done under aseptic condition in UV sterilized laminar flow.

All algae grown in 150 ml Erlenmeyer flasks containing 100 ml of culture medium were agitated twice a day in order to maintain them in actively growing stage. Subculturing was done after every 15 days of inoculation. The cultures were maintained in a culture chamber at the temperature of 22 $\pm$ 1°C and white light intensity of about 2 K lux for 16 hours a day.

PLATE - 1

Anabaena iyengarii Var. *tenuis* RAO

Figure A	Young vegetative filaments without any heterocyst and akinetes. X 400
Figure B	Vegetative filaments with heterocysts. X 400
Figure C	Vegetative filaments with heterocysts and young akinetes. X 400
Figure D	Vegetative filaments with mature akinetes. X 400
Figure E	Akinete germination. X 200
Figure F	Akinete germination. X 400

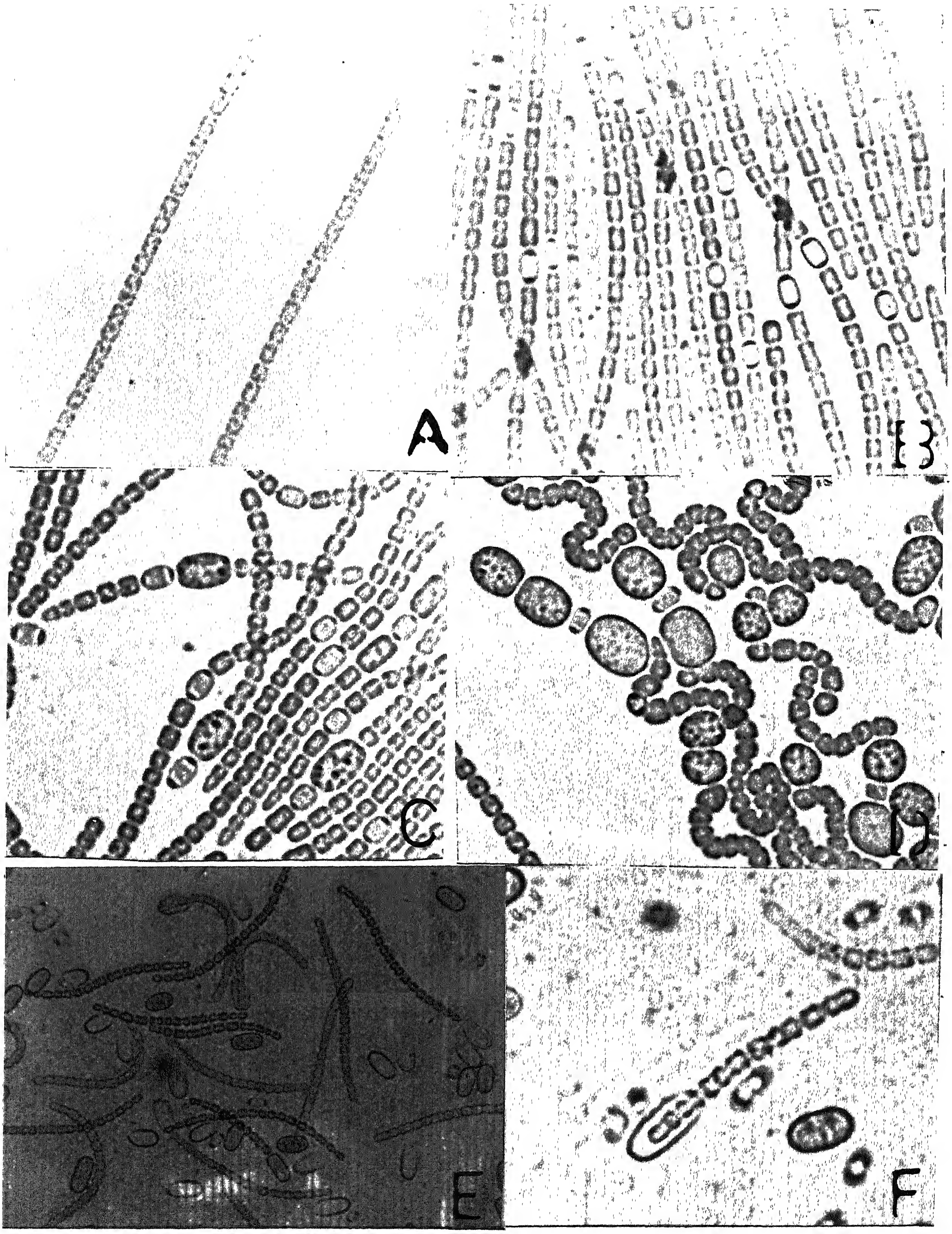


PLATE-1

PLATE - 2

Westiellopsis prolifica JANET

Figure A & B	Vegetative filaments showing branching. X 200, X 400
Figure C & D	Vegetative filaments showing young akinetes. X 400
Figure E	Vegetative filaments showing mature akinetes. X 400
Figure F & G	Akinete germination. X 400

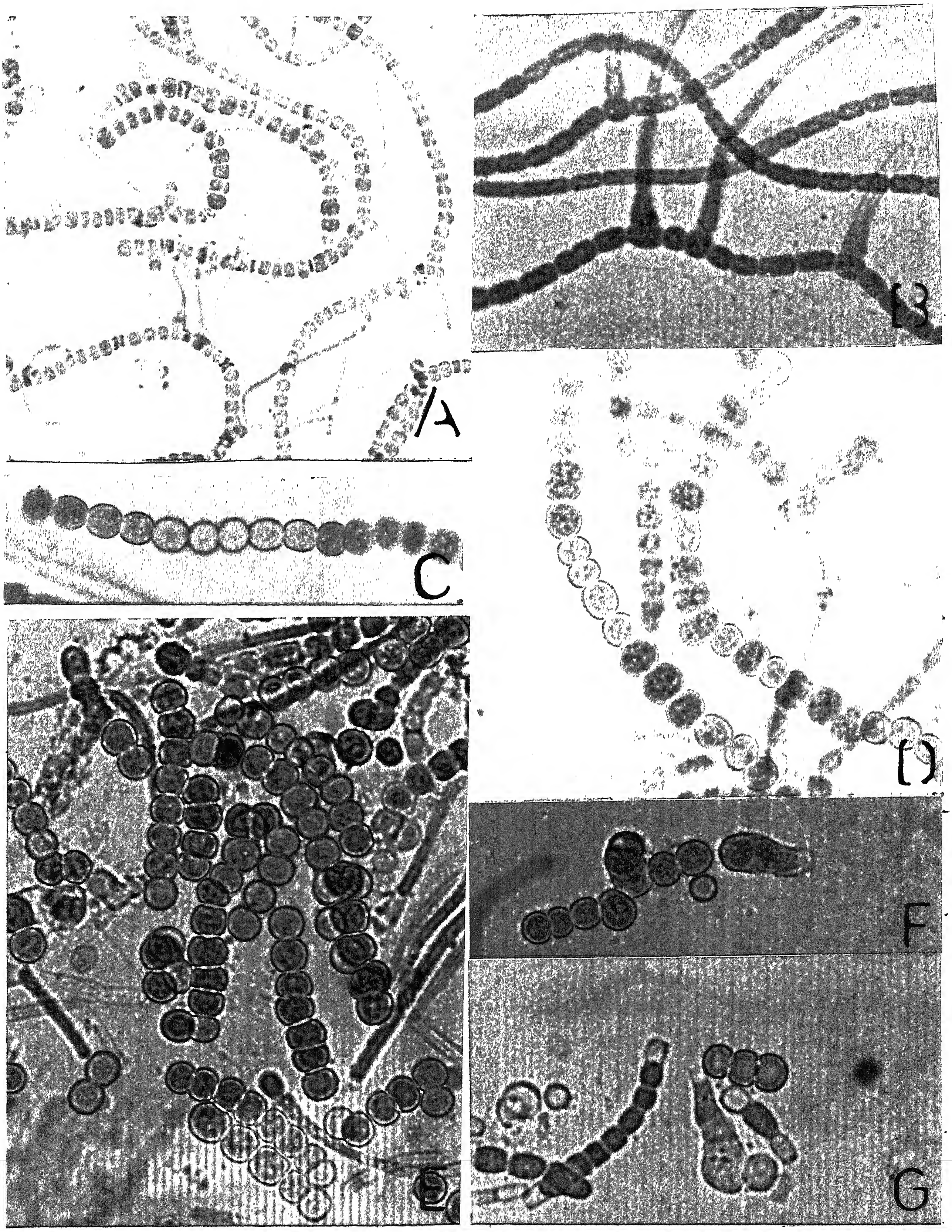


PLATE-2

PLATE - 3

Nostochopsis lobatus WOOD

Figure A Vegetative filaments showing branching. X 200

Figure B Vegetative filaments showing branching and lateral heterocyst. X 400

Figure C Vegetative filaments showing branching and interalary heterocyst. X 400.

Figure D Vegetative filaments with mature akinetes. X 200

Figure E Vegetative filaments with intercalary heterocysts and terminal heterocyst and akinetes. X 400

Figure F Akinete germination. X 400

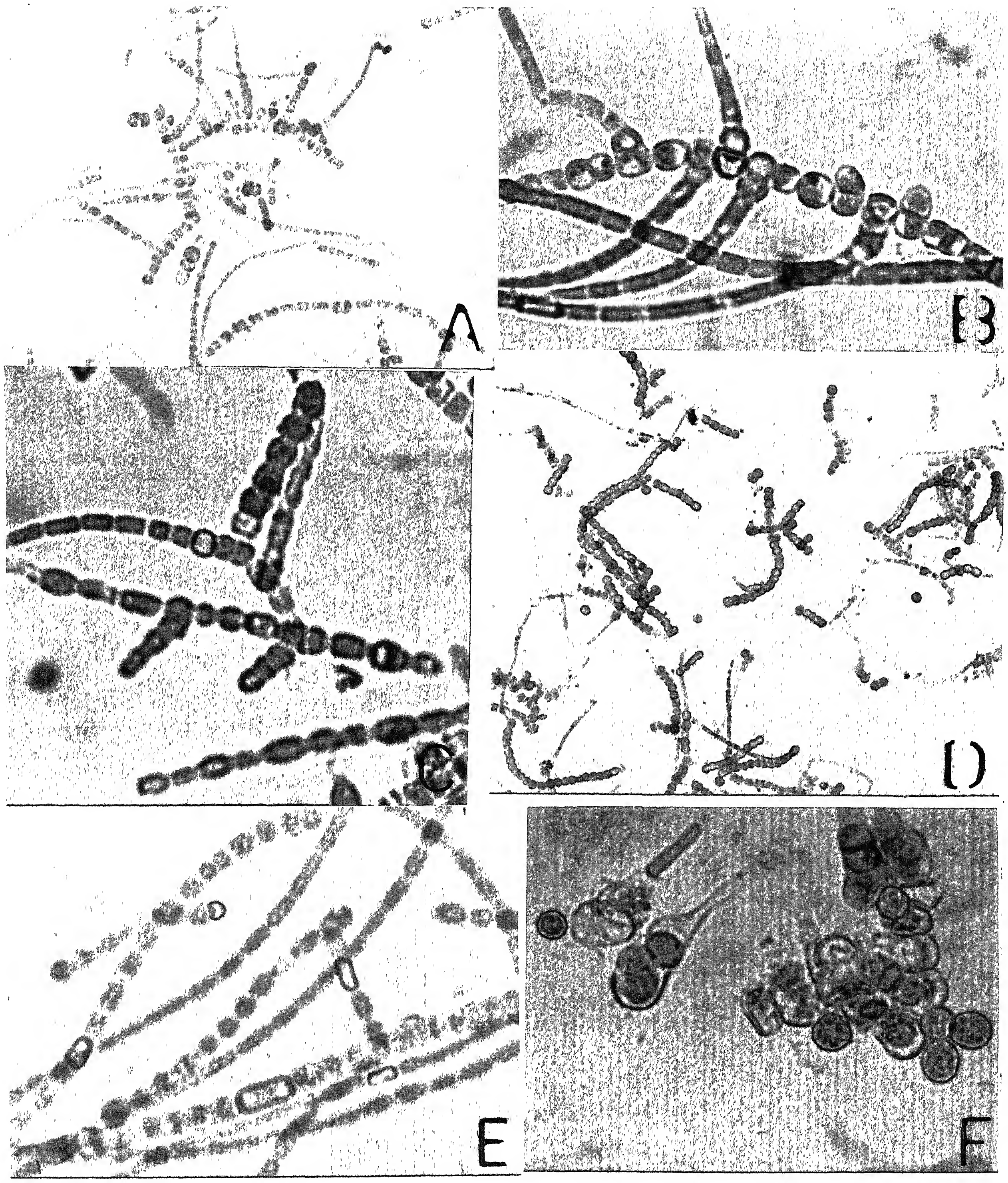


PLATE-3

PLATE - 4

Pithophora oedogonia (MONT.) WITTROCK

Figure A	Vegetative filament. X 50
Figure B	Vegetative filaments bearing akinetes. X 200
Figure C & D	Akinete germination. X 400

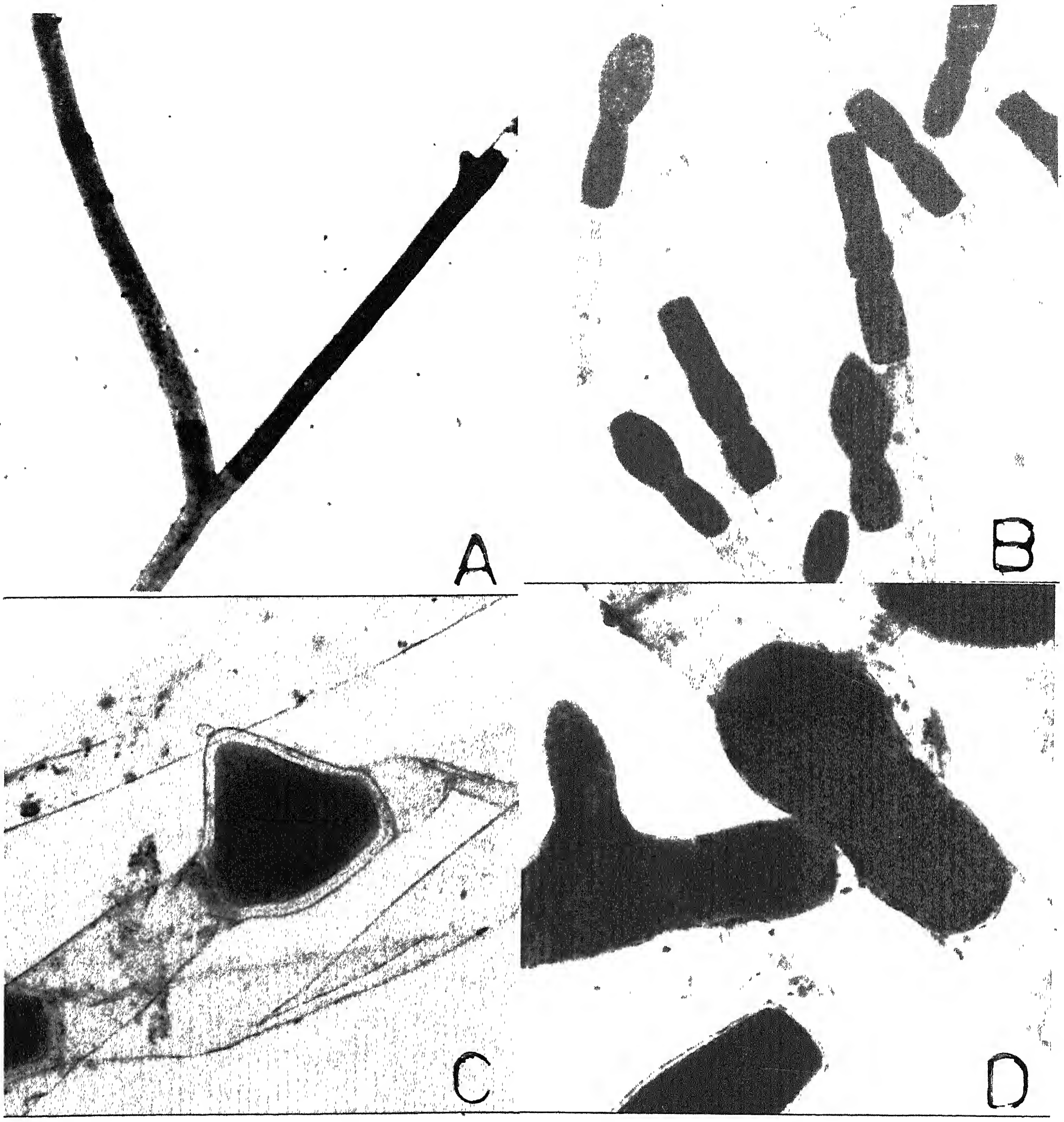


PLATE-4

PLATE - 5

Cladophora glomerata (L) KUETZING

Figure A Vegetative filaments. X 100

Figure B Vegetative filaments. X 200

Figure C Vegetative filament bearing zoosporangium. Inside zoosporangium zoospores are present. X 600

Figure D Zoospores germlings. X 400

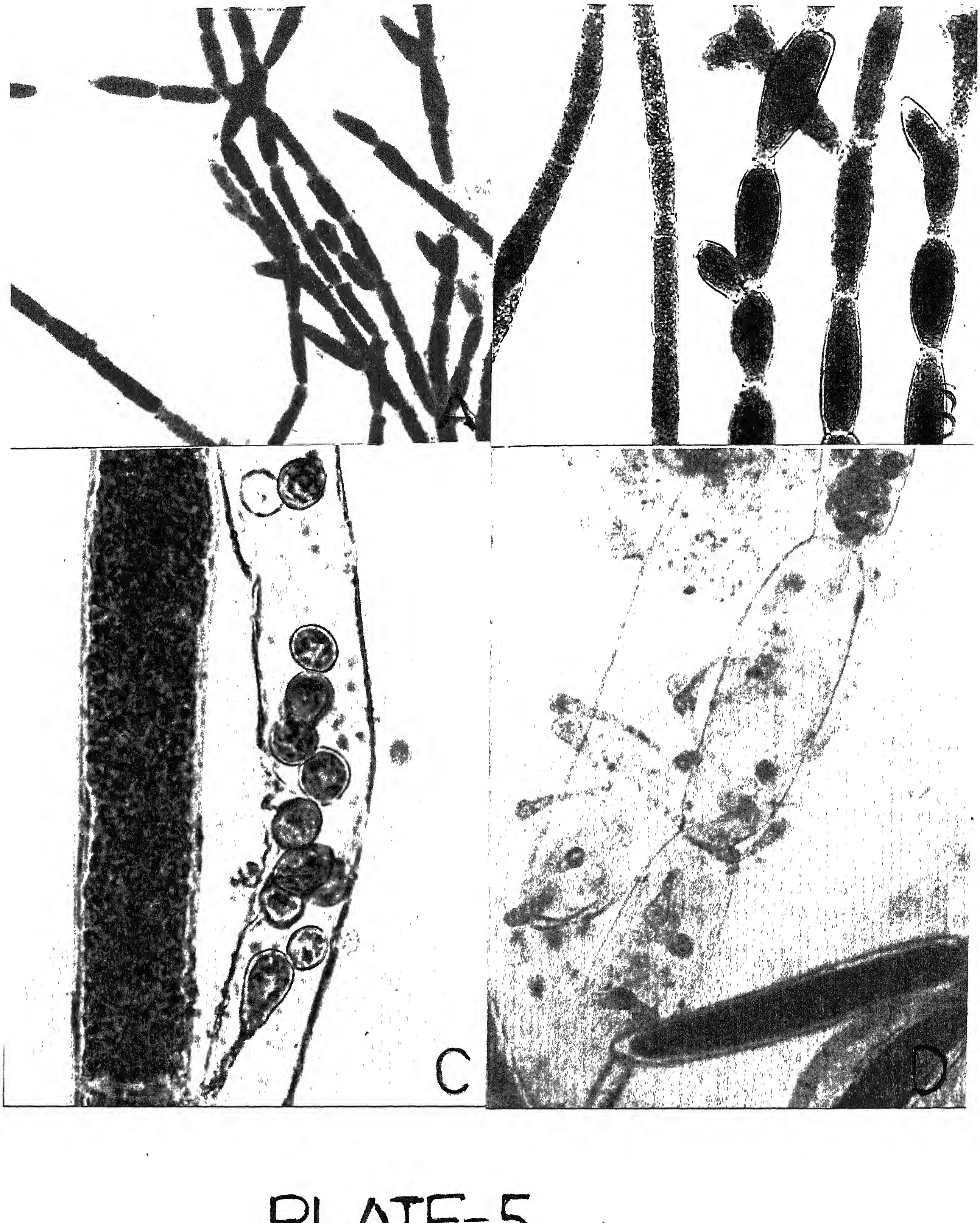


PLATE-5

PLATE - 6

***Rhizoclonium hieroglyphicum* (C.A.Ag.) KUETZING.**

Figure A Young filaments. X 100

Figure B Filament bearing zoosporangia. X 200

Figure C Zoosporangium with plenty of zoospores showing zoospore release. X 400

Figure D Release of zoospores one by one through a lateral pore. X 800

Figure E Zoospores germlings. X 400

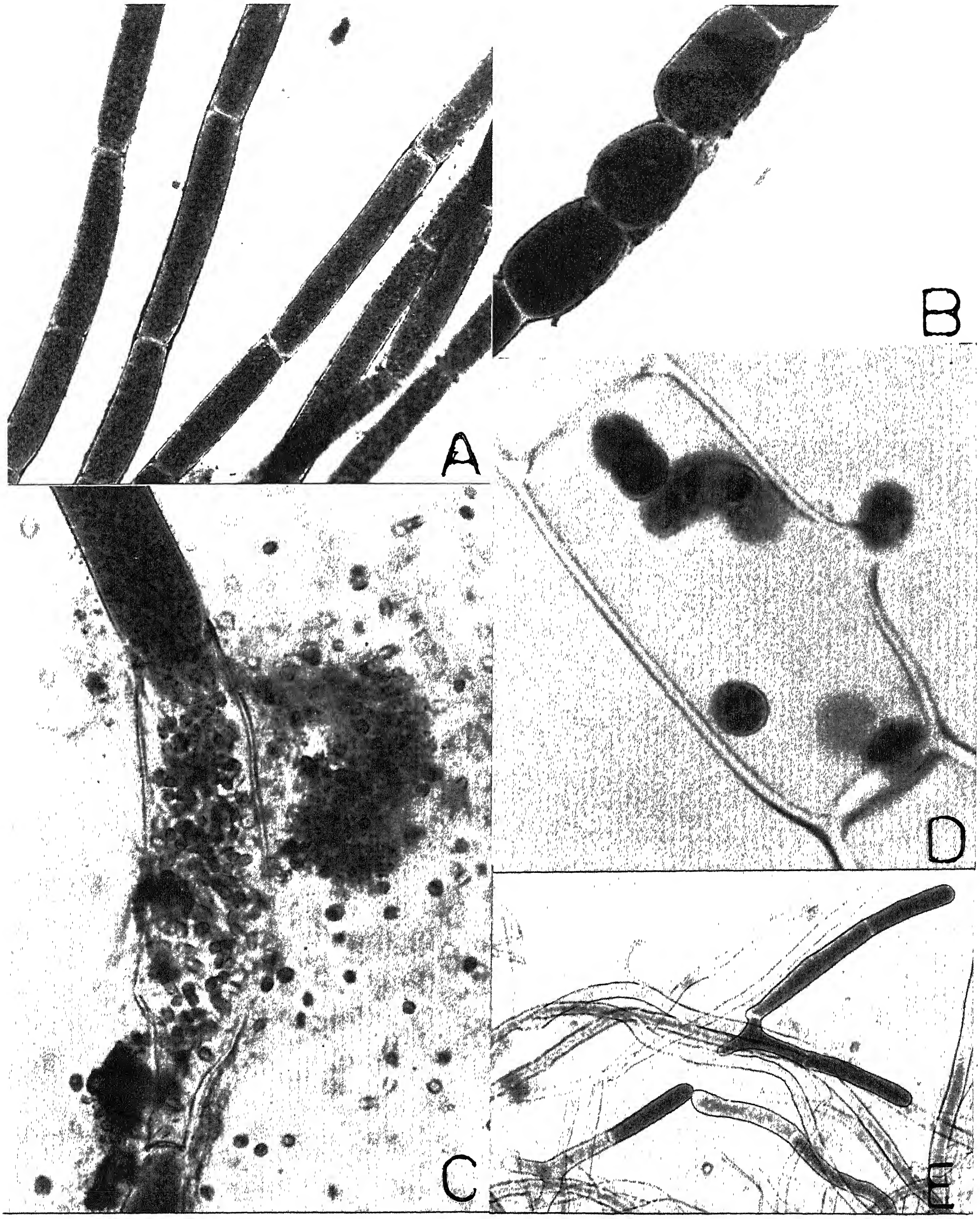


PLATE-6

CHAPTER III

EFFECTS OF NUTRIENTS PRESENT IN BG 11 MEDIUM ON THE SURVIVABILITY OF VEGETATIVE CELLS, DIFFERENTIATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITIY AND GERMINATION OF AKINETES OR ZOOSPORES

CHAPTER III

EFFECTS OF NUTRIENTS PRESENT IN BG 11 MEDIUM ON THE SURVIVABILITY OF VEGETATIVE CELLS, DIFFERENTIATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES

Very little information is available on the effects of nutrient concentration of different elements on the process of akinete or zoosporangium formation or akinete or zoospore germination in algae. Lack or limitation of phosphorus has been found to enhance akinete formation in *Cylindrospermum* sp. (Wolk, 1965; Reddy, 1976; Lang, 1980), *Aphanizomenon flos-aquae* (Gentile and Maloney, 1969), *Fischerella* sp. (Kaushik *et al.*, 1971), *Stigeoclonium* sp. (Martin & Whitton, 1987) and *Anabaena circinalis* (Van Dok and Hart, 1996); and that of nitrogen, the zoosporogenesis in *Ophiocystium* (Pecora and Russell, 1973) and akinete formation in *Gloeotrichia echinulata* (Wyman and Fay, 1986) and *Anabaena doliolum* (Rao *et al.*, 1987). Low levels of phosphate also induced akinete germination in *Nodularia spunigena* (Huber, 1985).

The deficiency of silicon induced auxospores formation in *Skeletonema costatum* (Davis *et al.*, 1973); and that of silicon and iron, the sporulation in *Eunotia soleirolii* (Von Stosch & Fecher, 1979). Media depleted of magnesium, calcium and sulphate suppressed akinete formation in *Gloetrichia echinulata* (Sinclair & Whitton, 1977). Presence of

calcium and magnesium induced pairing in *Mesotaenium* (Tiftickjian & Rayburn, 1986).

The present work was undertaken to see the effects of varying concentrations of nutrients present in BG 11 medium on the survivability of vegetative cells in all algae used; on the formation and germination of akinetes in *Anabaena iyengarii, Westiellopsis prolifica, Nostochopsis lobatus* and *Pithophora oedogonia*; and on the zoosporangium formation and germination of zoospores in *Cladophora glomerata* and *Rhizoclonium hieroglyphicum*. Viability of akinetes or zoosporangia formed under nutrients amendments was also determined.

METHODS

To test the responses of survivability of vegetative cells, the differentiation of akinetes and zoosporangia, and the germination of akinetes or zoospores in present algae to nutrients amendments of the standard medium, the BG 11 medium was altered either by omitting or increasing the concentration to 5 or 10 fold levels of that present in standard medium of either a particular nutrient compound in question or all of them in combination. The range of concentration of compounds varied between complete absence of all nutrient compounds (i.e. double distilled water) or that of a particular nutrient compound in question to progressive enhancement of that chemical to 5 or 10 fold level, or that of all of them to 5 or 10 fold levels as compared to that occurring in standard basal medium.

The pH of all amended media was adjusted to 7.5 prior to autoclaving as that of the standard BG 11 medium. Acid washed Borosil glass-wares properly rinsed with double distilled water were used throughout the present study.

(i) SURVIVABILITY OF VEGETATIVE CELLS, DIFFERENTIATION OF AKINETES AND ZOOSPORANGIA AND THEIR VIABILITY

Actively growing young vegetative filaments of all algae, carefully washed with the medium of particular composition (so as to remove the nutrients of the standard basal medium that may have been carried with the materials) were separately inoculated into culture tubes containing that respective medium. They were then placed in the culture chamber under experimental conditions. Controls were maintained in standard basal medium. Survivability of vegetative cells in all algae; the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*, and that of zoosporangia in *C. glomerata* and *R. hieroglyphicum*, were estimated, respectively, by counting the percentages of dead cells (if any), akinetes, and zoosporangia relative to the total number of about 5000-6000 vegetative cells at various time intervals of 15 to 60 days from the time of inoculation.

(ii) VIABILITY OF AKINETES OR ZOOSPORANGIA FORMED

The viability of akinetes or zoosporangia formed in amended media was determined by harvesting akinetes or zoosporangia formed in that media after 60 and 45 day of inoculation, respectively, washed with distilled water and inoculated into basal medium and placed in culture

chamber. Akinetes or zoosporangia bearing filaments harvested from basal medium inoculated similarly served as controls. The percentage germination of akinetes or the percentage of empty zoosporangia which have released zoospores, out of total akinetes or zoosporangia inoculated was estimated on 15 day of inoculation.

(iii) GERMINATION OF AKINETES AND ZOOSPORES

Mature akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and freshly released zoospores of *C. glomerata* (adhering to cover slips) and *R. hieroglyphicum* (adhering to parent filaments) formed in standard basal medium, washed carefully with the medium of particular composition, were separately inoculated into culture tubes containing that respective medium. They were then placed in culture chamber under culture conditions. Controls were maintained in standard basal medium. The percentage germination of akinetes and of zoospores was determined by counting about 2500-3000 akinetes or zoospores on 15 days of inoculation. The emergence of protuberance was taken as a criterion for germination.

RESULT AND DISCUSSION

A. OMISSION OF NUTRIENTS

(i) On vegetative survival and formation of akinetes and zoosporangia and their viability

Omission of different single nutrient compounds from the medium either did not effect or decreased slightly at various levels the survivability of vegetative cells in all algae used. But omission of all nutrient

compounds from the medium (i.e. double distilled water) certainly decreased the survivability of vegetative cells of all algae used much more than that in standard medium, and in this respect green algae used were more sensitive than blue-green algae (Table IA, Graph I). Green alga *P. oedogonia* was comparatively more sensitive to nutrients shortage than were other algae used (Table IA, Graph I). It is well known that algae may contain substantial intracellular nutrient reserves enabling them to survive and to form differentiated cells for long time survival. Blue-green algae are able to accumulate phosphorus far in excess of their needs (Fogg *et al.*, 1973; Whitton, 1973) and can also fulfil their nitrogen requirement by fixing atmospheric nitrogen.

An unavailability of different single nutrient compounds of the culture medium either did not affect much or decreased slightly at various levels the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and zoosporangia in *C. glomerata* and *R. hieroglyphicum*, but that of all nutrient compounds altogether, delayed and decreased drastically at various levels the formation of akinetes and zoosporangia in different algae used as compared to controls (Table IA, Graph I).

Low levels of nitrogen also decreased conjugation in *Zygnema circumcarinatum* (Jost, 1953), mating in *Chlamydomonas moewusii* (Bernstein & John, 1955; Trainor, 1959), zoosporogenesis in *Protosiphon botryoides* (O'Kelley & Deason, 1962), *Botrydiopsis sp., Bumilleriopsis sp.* and *Pseudollumilleriopsis sp.* (Pecora & Russell, 1973), and in *Pandorina*

unicocca (Rayburn, 1974), and akinete formation in *Nodularia spumigena* (Pandey & Talpasayi, 1980), *Chlorococcum echinozygotum* (O'Kelley, 1984) and *C. monoica* (Van Den Ende *et al.*, 1992).

In the present study, the formation (to a reduced extent) of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*, and of zoosporangia in *C. glomerata* and *R. hieroglyphicum* in media depleted of all nutrients indicate that these algae require very low amount of nutrients for the survivability and differentiation of reproductive cells. Waters of many lakes, as that of Wisconsin lakes are reported to be so dilute as that of rain water (Hutchinson, 1957) and the algal flora of such lakes must be growing and reproducing there at very low nutrients content.

In present study, the formation of zoosporangia in *C. glomerata* and *R. hieroglyphicum* was comparatively more sensitive to nutrients shortage than was akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* (Table IA, Graph I). Viability of akinetes or zoosporangia formed in medium containing no nutrients was severely affected.

(ii) On Germination of akinetes and zoospores

An unavailability of different single nutrient compounds or more distinctly that of all in combination from the basal medium decreased the germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and that of zoospores in *C. glomerata* and *R. hieroglyphicum* (Table IC, Graph I). The akinete germination in *Anabaena fertilissima* and *Anabaena arnoldii* was also retarded in media deficient in phosphate or

nitrate (Reddy, 1976), while that in *Fischerella muscicola* was not changed in media with or without phosphate (Kaushik *et al.*, 1971). According to Imahori and Iwasa (1965) inorganic basal medium even at half strength suppressed the germination of oospores of certain charophytes.

In the present study, an ability of algal akinetes or zoospores to germinate (albeit to a reduce extent) even in medium omitted of all nutrients indicate that they require very low amounts of extrabiotic nutrients to initiate the process of germination and probably the amount of nutrients present in standard BG 11 medium was sufficient to satisfy the nutritional need required by them.

B. Excess of Nutrients

(i) On vegetative survival and akinete or zoosporangium formation and their viability

In the present study, the media containing either 5 or 10 fold level of different single nutrient compound in question or more particularly that containing 5 or 10 fold level of all of them in combination also decreased in increasing order the survivability of vegetative cells in all algae used, the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*, and that of zoosporangia in *C. glomerata* and *R. hieroglyphicum* (Table IB, Graph II & III).

A. iyengarii was the most sensitive alga to nutrients abundance, since it was not tolerating much a medium containing 5 fold level of any of single nutrient compound in question (Table IB, Graph II). Most of *P.*

oedogonia vegetative cells also died without forming any akinete within 15 days of inoculation in medium containing 5 fold level of all nutrient compounds (Table IB, Graph II). However *W. prolifica* and *N. lobatus* were more or less equally most tolerant algae followed by *C. glomerata* and *R. hieroglyphicum* to high nutrients levels of medium (Table IB, Graph II & III). High levels of nutrients also affected viability of akinetes and zoosporangia very much (Table IB).

Increased levels of trace elements in the medium inhibited akinete formation in *Stigeoclonium pascheri* (Agrawal & Sarma, 1982b). Akinete formation in *P. oedogonia* was inhibited in presence of nickel and mercuric chloride (Chaudhary & Singh, 1986).

It was observed by Hsiao and Druehl (1973) that production of oogonia in gametophytes of *Laminaria saccharina* required a very limited amounts of nitrate and phosphate in the medium. Present study indicates that an enhancement in the quantity of different nutrients to 5 or 10 fold level of that present in the standard medium did not improve but proved inhibitory to both vegetative survival as well as formation of reproductive organs in all algae used, and this might probably be due to toxicity produced by high levels of nutrients. Thus the amount of nutrients present in standard BG 11 medium was sufficient to satisfy the nutritional need required for optimum achievement of vegetative survival and viable reproductive organs formation in all algae used.

(ii) On akinete and zoospore germination

The akinete germination in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and zoospore germination in *C. glomerata* and *R. hieroglyphicum* all decreased variously in ascending order with an increase in concentration of even a single nutrient compound of BG 11 medium to 5 and 10 fold level of that present in standard medium (Table ID, Graph II & III).

The zoospore germination in *C. glomerata* and *R. hieroglyphicum* was comparatively more sensitive to enhanced levels of nutrients content of the medium than akinete germination in *A. iyengarii, W. protifica, N. lobatus* and *P. oedogonia* (Table ID, Graph II & III).

Table I A : The percentage formation of dead cells (D) in all algae, akinetes (A) in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and Zoosporangia (Z) in *C. glomerata* and *R. hieroglyphicum* in media omitted of either single nutrient compound in question or all of them in combination.[a]

D, A, Z %	Days of inoculation	Control medium	Omission of							
			$NaNO_3$	K_2HPO_4	$MgSO_4 \cdot 7H_2O$	$CaCl_2 \cdot 2H_2O$	Na_2CO_3	Ferric ammonium citrate	Trace metal mix	All nutrient compounds
					A. iyengarii					
D	30	1	2	4	2	0	5	2	0	2
	60	2	3	6	5	2	7	6	4	5
A	30	20	5	15	20	20	12	20	25	5
	60	87	65[b]	40[b]	60[b]	60[b]	70[b]	70[b]	42[b]	10[c]
					W. prolifica					
D	30	0.5	1	6	2	0.5	0.3	0.4	0.6	7.0
	60	3	12	8	3	1	0.6	0.5	1.0	15
A	30	48	47	40	38	18	26	15	23	20
	60	81	53[b]	69[b]	67[b]	70[b]	60[b]	68[b]	68[b]	30[c]
					N. lobatus					
D	30	0.5	1	5	2	0.5	0.5	1	0.5	0.5
	60	1	2	6	2	1	1	1	1	16
A	30	56	53	55	50	17	27	34	28	16
	60	86	80[b]	75[b]	71[b]	90[b]	69[b]	50[b]	85[b]	35[c]

					P. oedogonium					
D	15	1	0	4	11	0	0	2	3	42
	45	7	4	5	13	13	9	11	10	68
A	15	12	0	2	10	0	0	2	5	1
	45	21	4[b]	7[b]	11[b]	2[b]	6[b]	7[b]	10[b]	3[c]
					C. glomerata					
D	15	3	0	0	0	0	0	0	0	20
	45	4	6	9	5	2	3	7	4	40
Z	15	8	0	0	0	0	0	0	0	0
	45	20	11[b]	15[b]	10[b]	12[b]	7[b]	7[b]	5[b]	4[c]
					R. hieroglyphicum					
D	15	2	0	0	2	0	0	0	0	5
	45	5	4	8	9	3	2	6	1	20
Z	15	14	0	0	14	0	0	0	0	0
	45	36	11[b]	10[b]	25[b]	30[b]	18[b]	16[b]	18[b]	8[c]

[a]Data represent rounded mean value of three replicates.
[b]Viability 20-30% as compared to those formed in control medium.
[c]Viability 10-15% as compared to those formed in control medium.
[d]No viability at all.

Table I B : The percentage formation of dead cells (D) in all algae, akinetes (A) in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and Zoosporangia (Z) in *C. glomerata, R. hieroglyphicum* in media containing 5 or 10 fold level of either a single nutrient compound or all of them together[a]

D, A, Z %	Days of inoculation	Control medium	Fold level															
			$NaNO_3$		K_2HPO_4		$MgSO_4 \cdot 7H_2O$		$CaCl_2 \cdot 2H_2O$		$NaCO_3$		Ferric ammonium citrate		Trace metal mix		All nutrient compounds	
			5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10
							A. iyengarii											
D	30	0	0	0	0	0	0	0	0	4	0	0	4	2	2	5	5	10
	60	2	50	50	65	90	55	75	73	81	60	75	68	85	70	75	80	90
A	30	25	20	20	21	5	30	20	17	9	40	15	34	5	15	10	10	5
	60	87	50[b]	35[c]	40[b]	10[c]	45[b]	25[c]	27[b]	19[c]	40[b]	25[c]	42[b]	15[c]	30[b]	20[c]	20[c]	10[c]
							W. prolifica											
D	30	0.5	1	2	2	2	2	3	0	0.5	0	0	0.5	0	0	0	12	20
	60	3	5	2	7	5	3	5	1	2	0.4	0.5	1	1	1	1	35	90
A	30	48	57	52	33	57	59	61	24	25	37	26	12	9	26	17	11	10
	60	81	60[b]	64[c]	71[b]	73[c]	63[b]	62[c]	79[b]	80[c]	75[b]	71[c]	56[b]	49[c]	57[b]	51[c]	65[c]	10[c]
							N. lobatus											
D	30	0.5	1	1	1	1	1	1	1	1	0	0	1	1	0.5	0.5	10	30
	60	1	2	3	2	2	2	3	1	2	0.5	1	2	2	1	1	30	44
A	30	56	49	45	53	69	73	60	22	25	18	19	56	43	25	19	15	10
	60	86	79[b]	75[c]	70[b]	83[c]	78[b]	80[c]	87[b]	85[c]	58[b]	65[c]	70[b]	75[c]	82[b]	78[c]	40[c]	20[c]

								P. oedogonia											
D	15	1	0	2	5	10	11	8	0	0	0	0	10	15	4	5	87	99
	45	7	9	12	15	16	18	16	20	26	9	25	93	98	6	25	97	99
A	15	3	0	0	6	2	1	7	0	0	0	0	0	0	0	0	3	1
	45	21	6^b	9^c	8^b	10^c	6^b	13^c	5^b	1^c	9^b	5^c	7^b	2^c	2^b	4^c	3^c	1^c
								C. glomerata											
D	15	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	20	15
	45	4	7	10	9	12	5	7	10	26	4	8	10	20	7	12	40	80
Z	15	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	20	15b	15^c	9^b	6^c	8^b	5^c	11^b	9^c	10^b	6^c	12^b	7^c	11^b	7^c	4^c	2^c
								R. hieroglyphicum											
D	15	2	0	0	0	0	4	5	0	0	0	0	0	0	0	0	30	35
	45	4	10	15	12	19	9	13	10	8	3	7	5	9	3	10	50	60
Z	15	14	0	0	0	0	2	5	0	0	0	0	0	0	0	0	0	0
	45	37	20^b	10^c	25^b	14^c	27^b	24^c	25^b	19^c	10^b	7^c	16^b	10^c	5^b	3^c	4^c	2^c

[a]Data represent rounded mean value of three replicates.
[b]Viability 20-35% as compared to those formed in control medium.
[c]Viability 5-15% as compared to those formed in control medium.

Table I C : Percentage germination of akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and zoospores of *C. glomerata* and *R. hieroglyphicum* in media omitted of either a single nutrient compound or all of them together on 15 days of inoculation[a]

Algae	Control medium	Omission of							
		$NaNO_3$	K_2HPO_4	$MgSO_4 \cdot 7H_2O$	$CaCl_2 \cdot 2H_2O$	Na_2CO_3	Ferric ammonium citrate	Trace metal mix	All nutrient compounds
A. iyengarii	55	35	40	34	37	34	33	38	15
W. prolifica	55	40	32	44	45	49	50	39	25
N. lobatus	60	45	50	50	34	42	35	32	23
P. oedogonia	70	30	33	40	44	40	41	46	25
C. glomerata	50	15	28	23	29	20	27	24	20
R. hieroglyphicum	60	20	23	30	36	27	30	34	24

[a]Data represent rounded mean value of three replicates.

Table I D : Percentage germination of akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and of zoospores of *C. glomerata* and *R. hieroglyphicum* in media omitted of either 5 or 10 fold level of a single nutrient compound or all of them together as compared to standard medium on 15 days of inoculation[a]

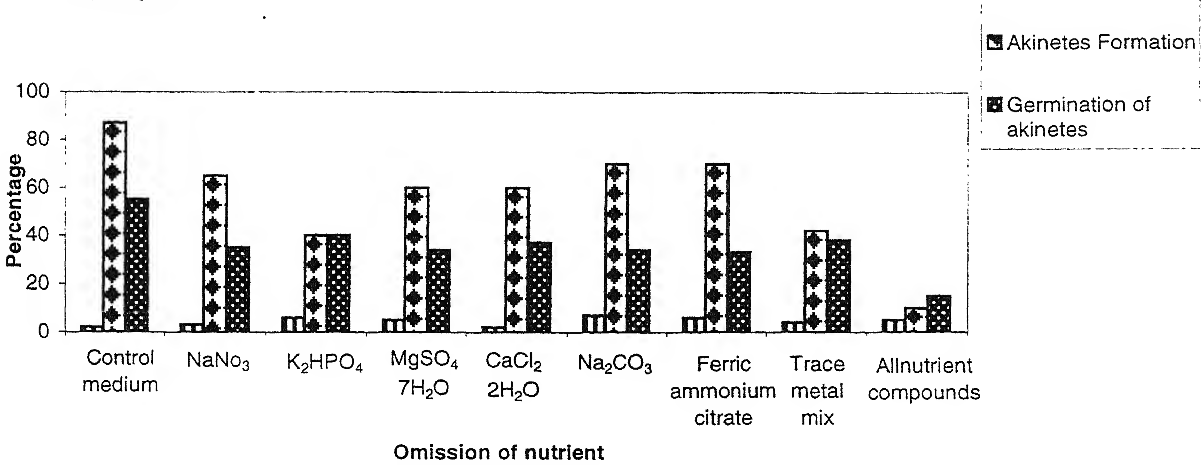
Algae	Control medium	Fold level															
		$NaNO_3$		K_2HPO_4		$MgSO_4 \cdot 7H_2O$		$CaCl_2 \cdot 2H_2O$		$NaCO_3$		Ferric ammonium citrate		Trace metal mix		All nutrient compounds	
		5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10
A. iyengarii	50	20	15	18	10	23	20	23	13	28	15	20	12	19	10	14	11
W. prolifica	55	25	20	10	9	30	25	30	20	25	12	30	25	25	17	15	10
N. lobatus	60	20	10	16	10	35	28	34	17	26	20	24	19	29	20	20	12
P. oedogonia	70	19	10	22	20	25	20	31	21	20	17	45	28	28	20	24	09
C. glomerata	50	17	05	15	10	18	10	22	08	13	10	24	17	15	10	20	08
R. hieroglyphicum	55	20	08	18	12	20	15	24	10	15	13	20	10	20	15	17	10

[a]Data represent rounded mean value of three replicates.

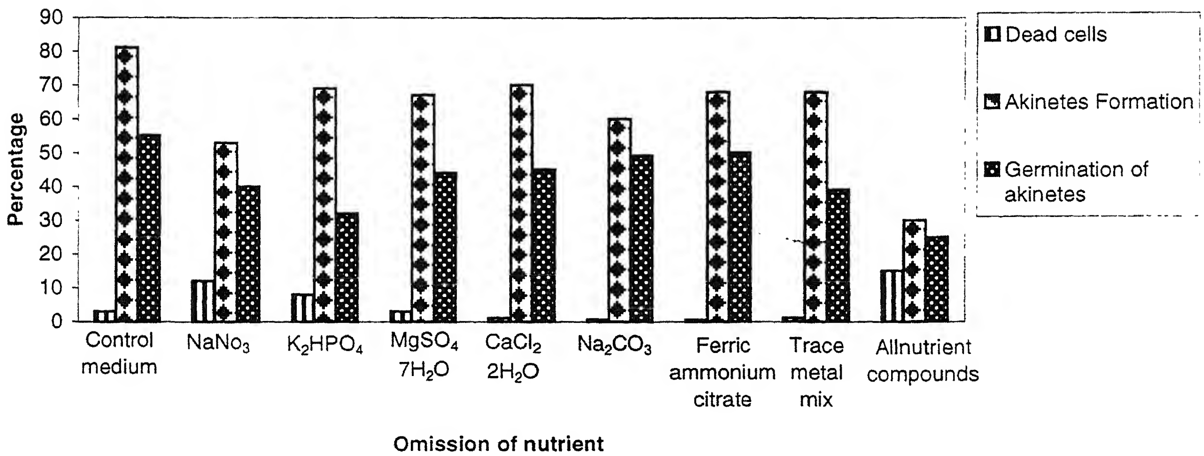
GRAPH- I

THE PERCENTAGE FORMATION OF DEAD CELLS IN ALL ALGAE, AKINETES OR ZOOSPORANGIA FORMATION AND GERMINATION OF AKINETES OR ZOOSPORES IN MEDIA OMITTED OF EITHERA SINGLE NUTRIENT COMPOUND IN QUESTION OR ALL OF THEM IN COMBINATION

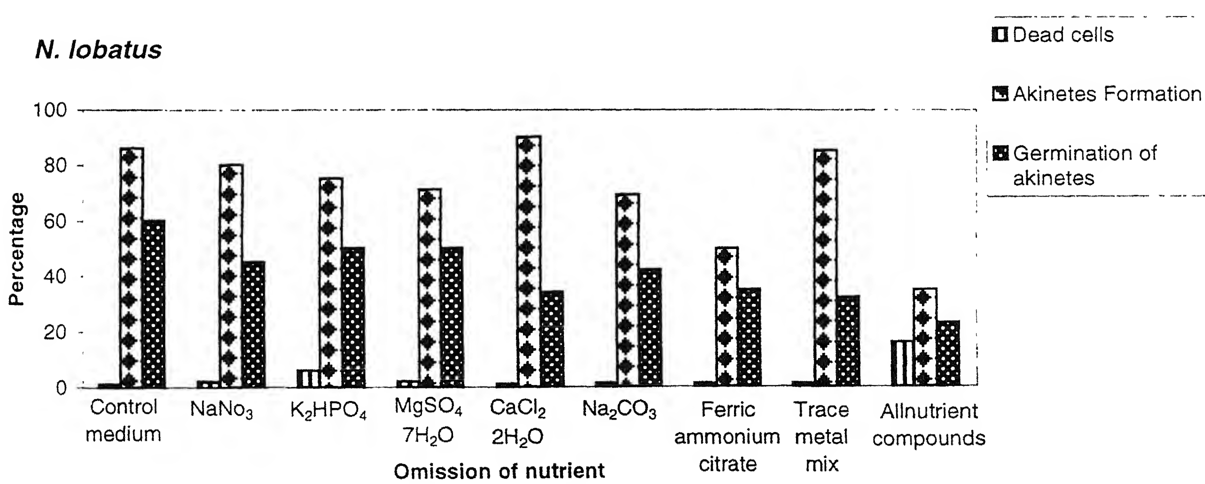
A. iyengarii



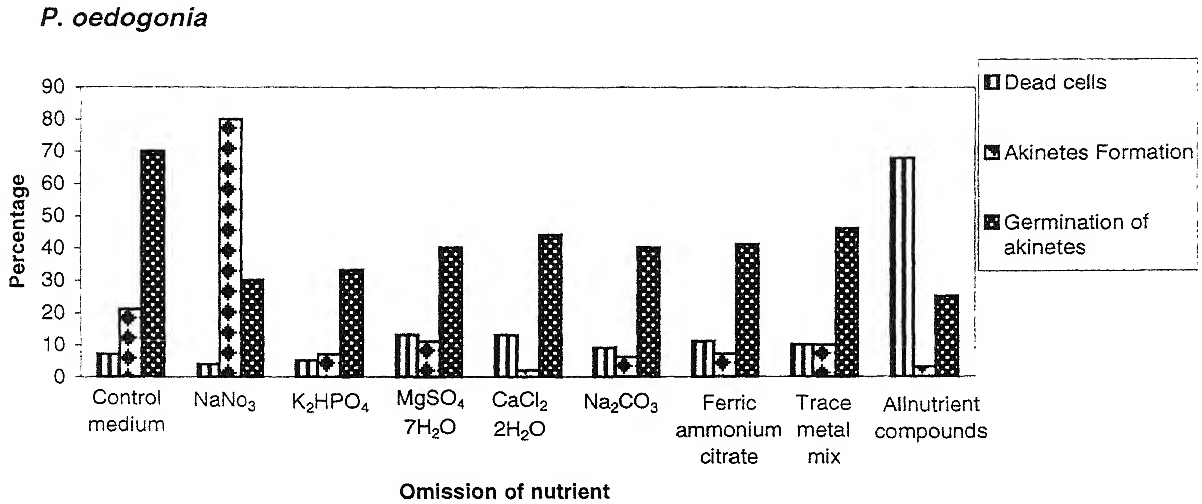
W. prolifica

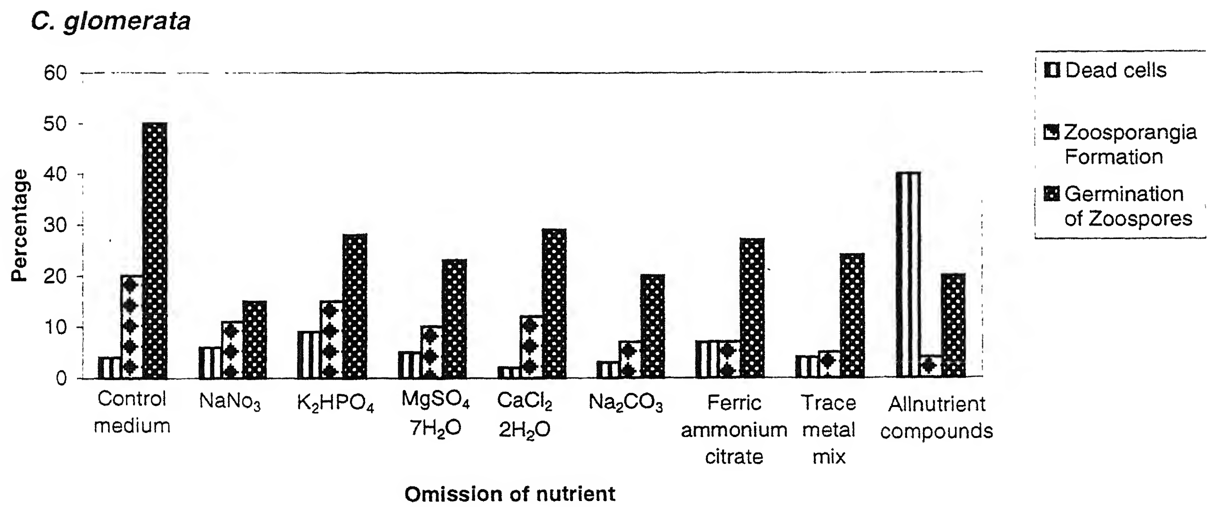


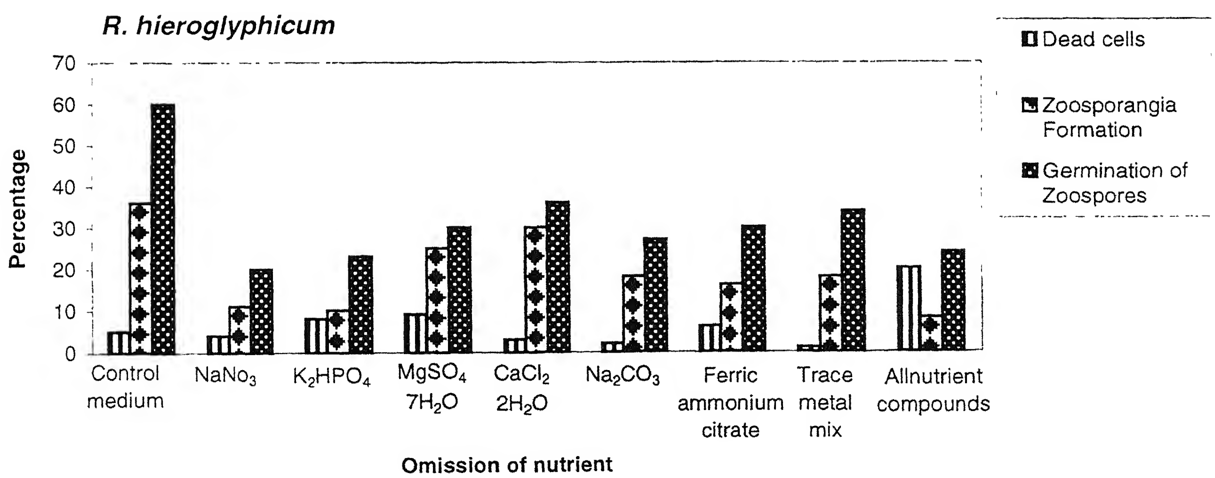
N. lobatus



Contd.

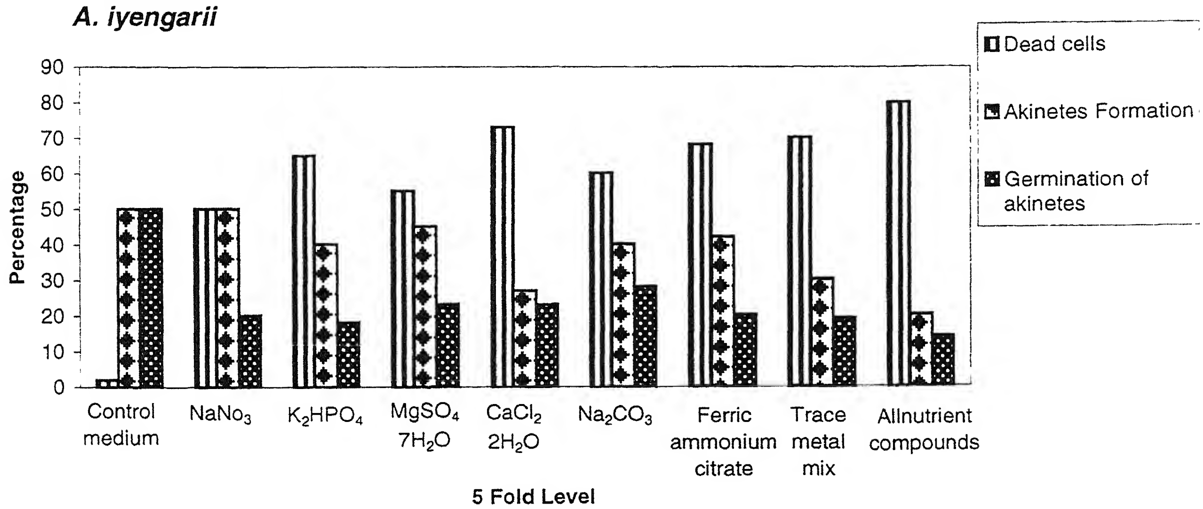

P. oedogonia
Percentage
90
80
70
60
50
40
30
20
10
0
Dead cells
Akinetes Formation
Germination of akinetes
Control medium
NaNo3
K2HPO4
MgSO4 7H2O
CaCl2 2H2O
Na2CO3
Ferric ammonium citrate
Trace metal mix
Allnutrient compounds
Omission of nutrient

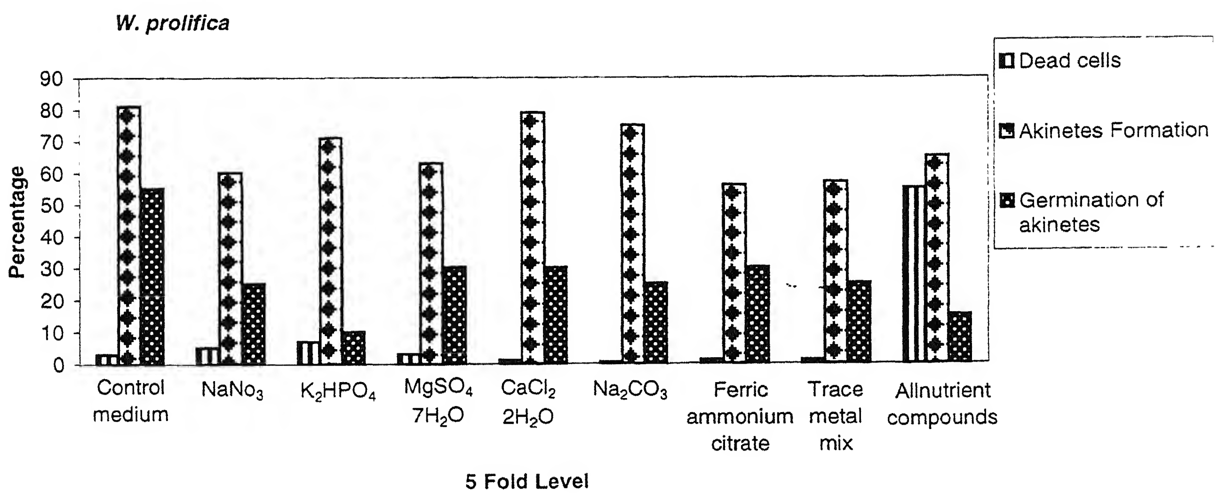

C. glomerata
Percentage
60
50
40
30
20
10
0
Dead cells
Zoosporangia Formation
Germination of Zoospores
Control medium
NaNo3
K2HPO4
MgSO4 7H2O
CaCl2 2H2O
Na2CO3
Ferric ammonium citrate
Trace metal mix
Allnutrient compounds
Omission of nutrient

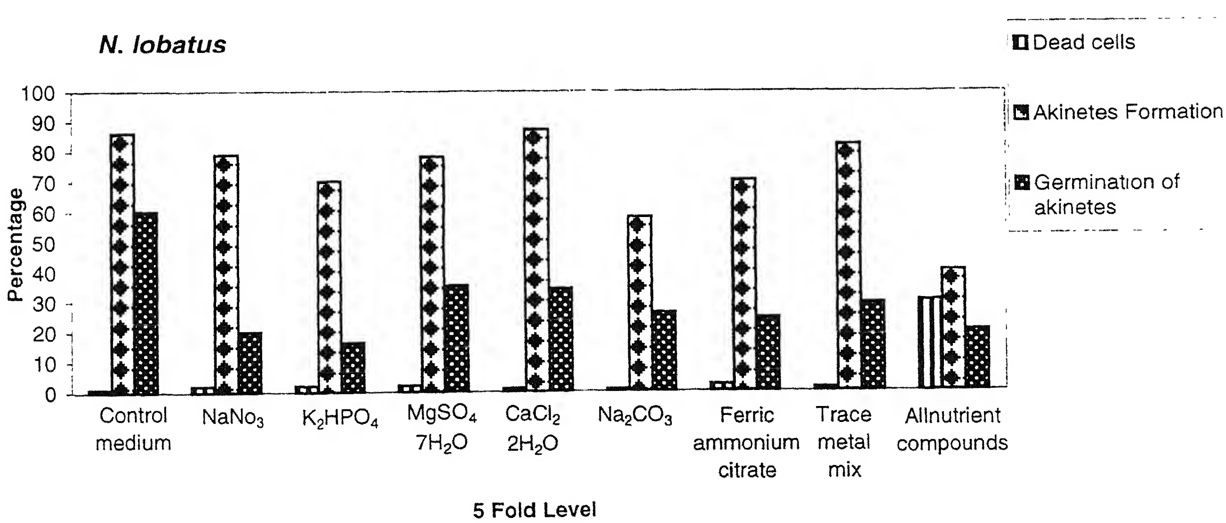

R. hieroglyphicum
Percentage
70
60
50
40
30
20
10
0
Dead cells
Zoosporangia Formation
Germination of Zoospores
Control medium
NaNo3
K2HPO4
MgSO4 7H2O
CaCl2 2H2O
Na2CO3
Ferric ammonium citrate
Trace metal mix
Allnutrient compounds
Omission of nutrient

GRAPH- II

THE PERCENTAGE FORMATION OF DEAD CELLS IN ALL ALGAE, AKINETES OR ZOOSPORANGIA FORMATION AND GERMINATION OF AKINETES OR ZOOSPORES IN MEDIA CONTAINING 5 FOLD LEVEL OF EITHERA SINGLE NUTRIENT COMPOUND OR ALL OF THEM IN COMBINATION

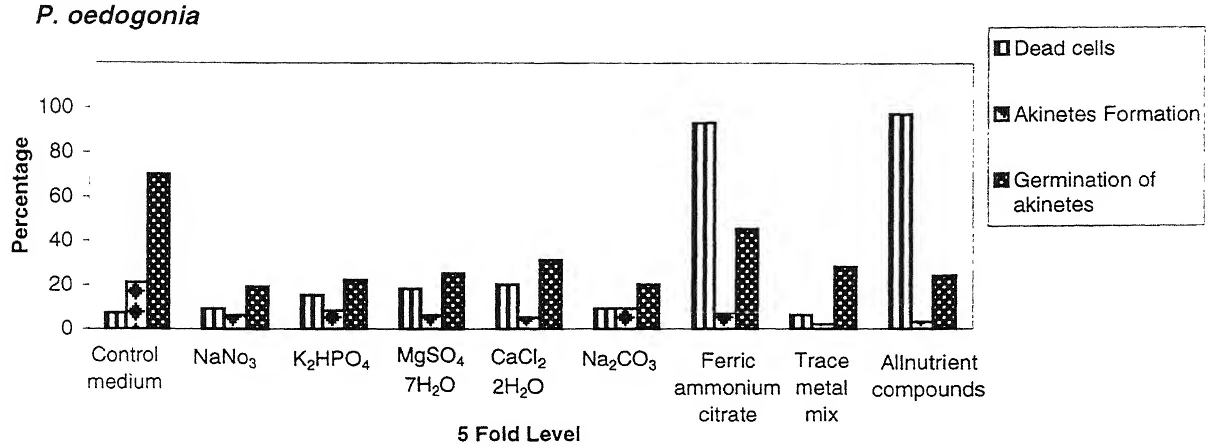




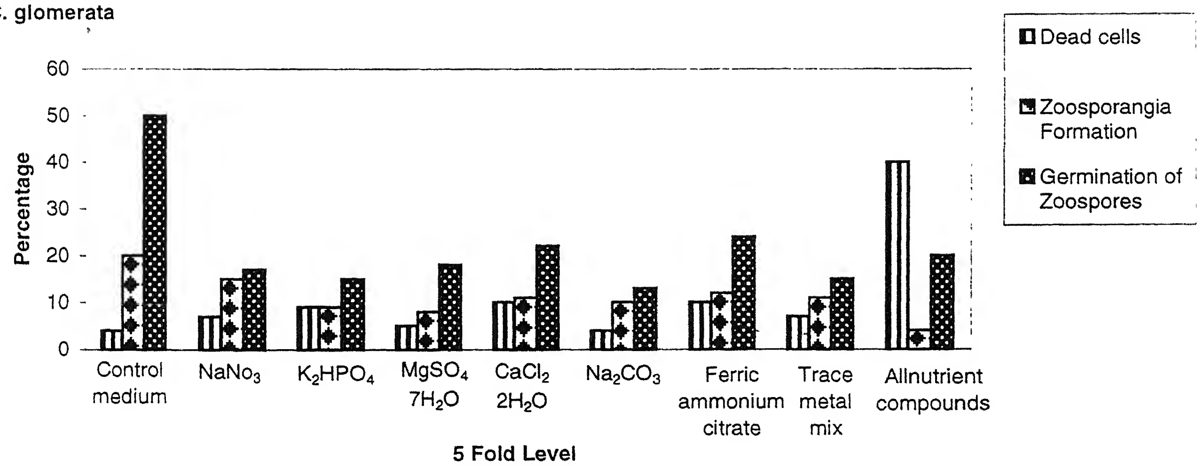


Contd.

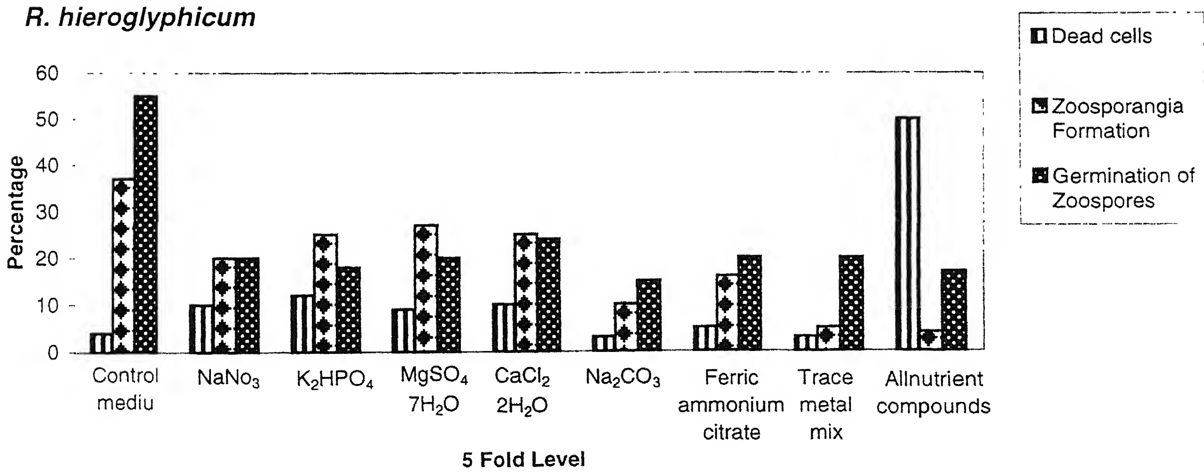
P. oedogonia



C. glomerata



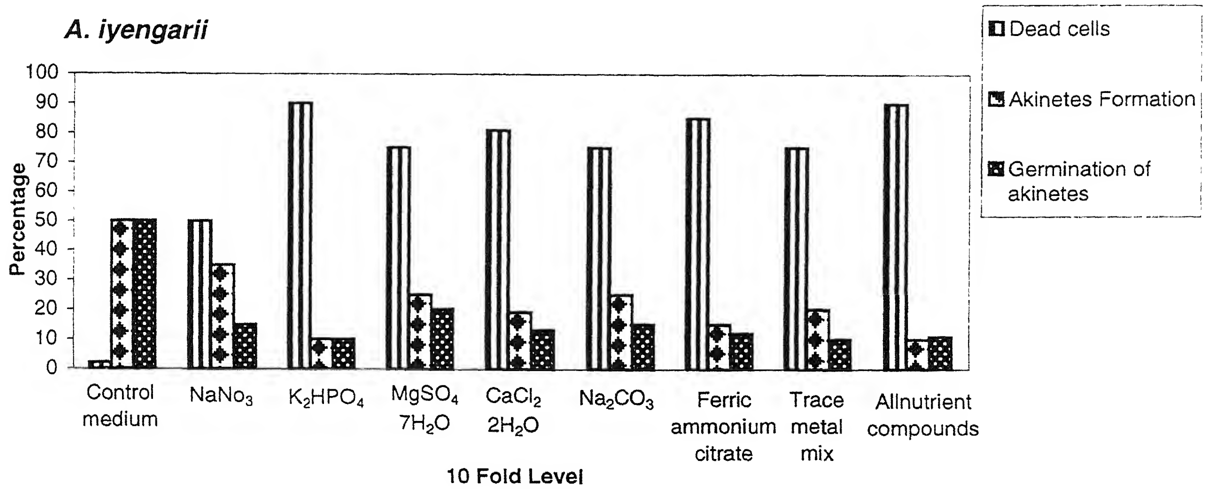
R. hieroglyphicum



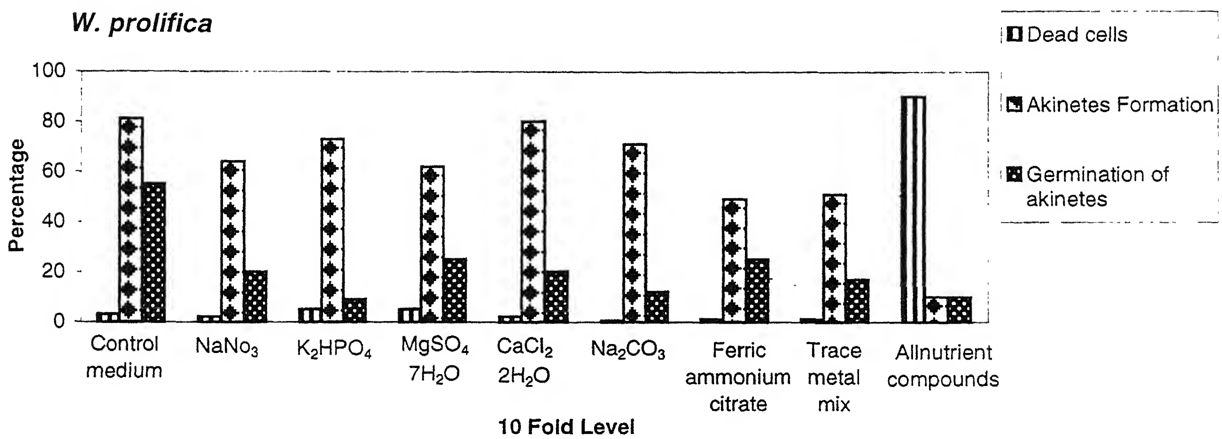
GRAPH- III

THE PERCENTAGE FORMATION OF DEAD CELLS IN ALL ALGAE, AKINETES OR ZOOSPORANGIA FORMATION AND GERMINATION OF AKINETES OR ZOOSPORES IN MEDIA CONTAINING 10 FOLD LEVEL OF EITHERA SINGLE NUTRIENT COMPOUND OR ALL OF THEM TOGETHER

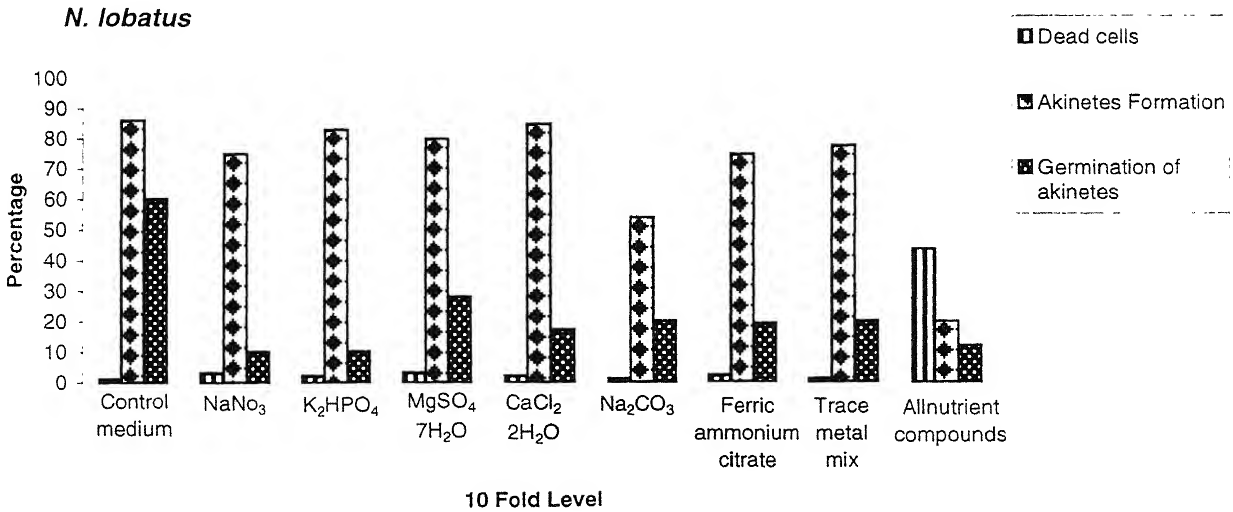
A. iyengarii



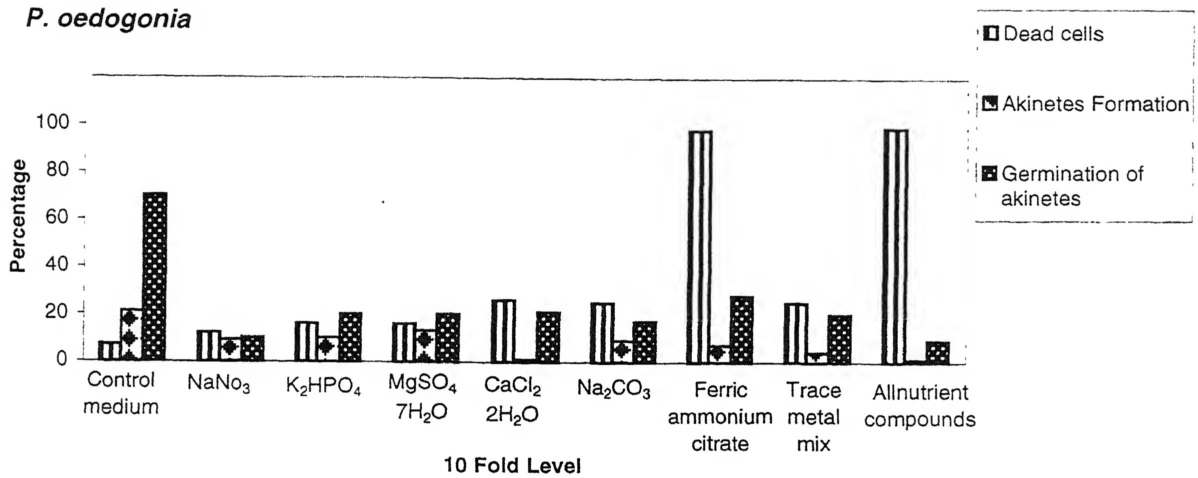
W. prolifica



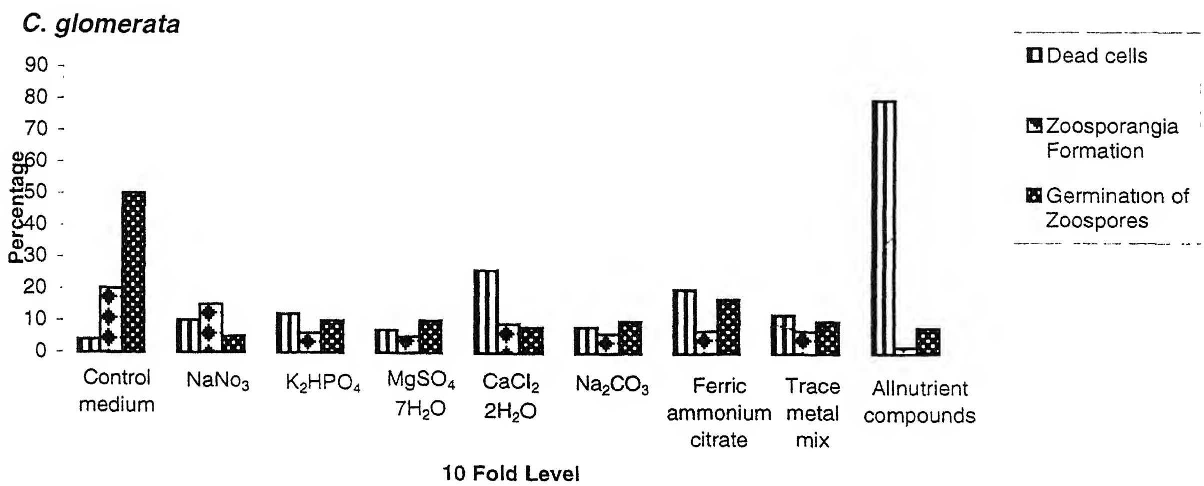
N. lobatus



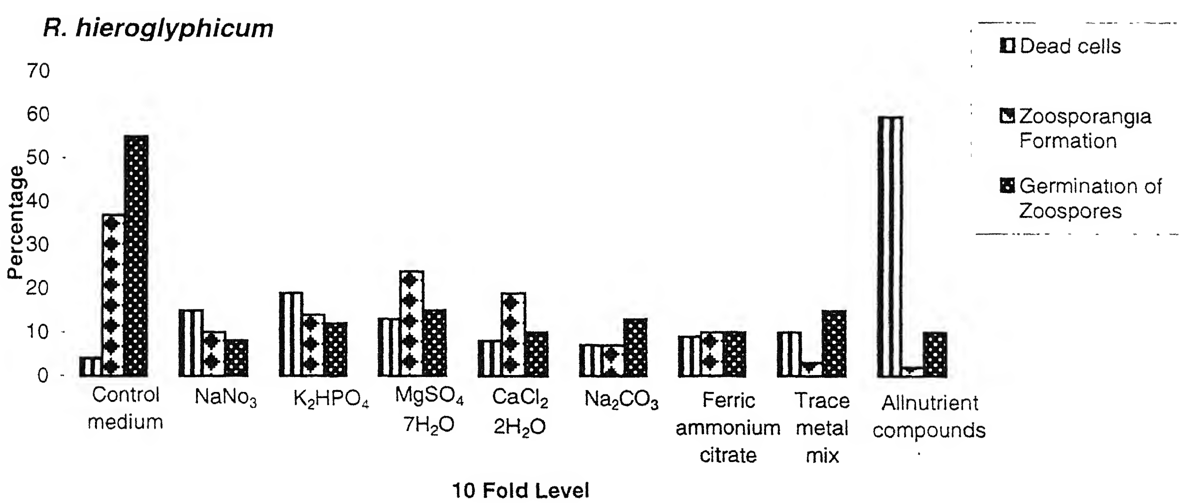
Contd..



P. oedogonia
Dead cells
Akinetes Formation
Germination of akinetes
Percentage
100
80
60
40
20
0
Control medium
NaNo3
K2HPO4
MgSO4 7H2O
CaCl2 2H2O
Na2CO3
Ferric ammonium citrate
Trace metal mix
Allnutrient compounds
10 Fold Level



C. glomerata
Dead cells
Zoosporangia Formation
Germination of Zoospores
Percentage
90
80
70
60
50
40
30
20
10
0
Control medium
NaNo3
K2HPO4
MgSO4 7H2O
CaCl2 2H2O
Na2CO3
Ferric ammonium citrate
Trace metal mix
Allnutrient compounds
10 Fold Level



R. hieroglyphicum
Dead cells
Zoosporangia Formation
Germination of Zoospores
Percentage
70
60
50
40
30
20
10
0
Control medium
NaNo3
K2HPO4
MgSO4 7H2O
CaCl2 2H2O
Na2CO3
Ferric ammonium citrate
Trace metal mix
Allnutrient compounds
10 Fold Level

CHAPTER IV

EFFECTS OF PH ON THE SURVIVABILITY OF VEGETATIVE CELLS, DIFFERENTIATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE USED

CHAPTER IV

EFFECTS OF PH ON THE SURVIVABILITY OF VEGETATIVE CELLS, DIFFERENTIATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE USED

Kretschmer (1930) first showed that pH of the culture medium on the alkaline side was suitable for the formation of zoospores or sex organs in *Oedogonium*. Formation of cysts in *Platymonas* (Tanoue and Aruga, 1975) and spores in Eunotia (Von Stosch and Fecher, 1979) was remarkably enhanced within very short period of time on raising the pH of the cultures to 9.0 or by lowering it to 6.0 as compared to their sluggish formation at the pH of 7.0 or 8.0. However, akinete formation in *Nodularia spumigena* (Pandey & Talpasayi, 1980), *Stigeoclonium pascheri* (Agrawal and Sarma, 1982a) and *Anabaena doliolum* (Pandey and Kashyap, 1987) was favoured at pH 8.0.

The present study incorporates the results of different pH values ranging between 3.5 to 11 on the survivability of vegetative cells of all algae used, on akinete formation and germination in *Anabaena iyengarii, Westiellopsis prolifica, Nostochopsis lobatus* and *Pithophora-oedogonia*, and on zoosporangium formation and zoospores germination in *Cladophora glomerata* and *Rhizoclonium hieroglyphicum*. Viability of akinetes or zoosporangia formed at different pH was also determined.

METHODS

The BG 11 medium was adjusted to different pH levels of 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 and 11.0 prior to autoclaving either by adding 1 N HCl or 2% NaOH solution. The pH of the medium was measured with help of a Toshniwal digital pH meter.

Survivability of vegetative cells and formation of akinetes and zoosporangia

Seven-day-old actively growing young vegetative filaments of all algae, washed with the medium of particular pH value, were inoculated, separately, into culture tubes containing that medium. They were then placed in culture chamber under experimental conditions. The survivability of vegetative cells was determined by counting number of dead vegetative cells, if any, with respect to total number of about 5000-6000 vegetative cells on 30 and 60 days of inoculation in *A. iyengarii, W. prolifica* and *N. lobatus* and on 15 and 45 days of inoculation in *P. oedogonia, C. glomerata* and *R. hieroglyphicum.*

In the present study, the pH of the inoculated culture medium got increased from 3.5 to 3.8, 4.0 to 4.3, 5.0 to 5.3, 6.0 to 6.4, 7.0 to 7.4 and 8.0 to 8.3 in 30 days old culture. However, no change was observed in the pH of the medium adjusted to either 10.0 or 11.0 even after 30 days of inoculation of experimental materials.

Viability of akinetes or zoosporangia formed

The viability of akinetes or zoosporangia formed was determined by harvesting akinetes and zoosporangia formed at different pH levels after

60 and 45 day of inoculation, respectively. They were washed with distilled water and inoculated into basal media and placed in culture chamber. The akinetes or zoosporangia harvested from pH 7 inoculated similarly served as controls. The percentage germination of akinetes or the percentage of empty zoosporangia which have released zoospores, out of total number of 2000-3000 akinetes of zoosporangia inoculated was estimated on 15 day of inoculation.

Germination of akinetes or zoospores

Mature akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and freshly released zoospores of *C. glomerata* and *R. hieroglyphicum*, formed in basal medium at pH 7.5, washed with the medium of different pH were separately inoculated into culture tubes containing medium of that pH. All inoculated culture tubes were placed in culture chamber. The percentage germination of akinetes or zoospores was determined by counting about 3000 akinetes or zoospores on 15 days after inoculation.

RESULTS AND DISCUSSION

Survivability of vegetative cells and formation of akinetes or zoosporangia and their viability

All vegetative cells of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* died without forming any akinete at pH 3.5 within 30, 60, 60 and 15 days of inoculation, respectively (Table IIA, Graph IV). However, vegetative cells of *C. glomerata* and *R. hieroglyphicum* could survive pH 3.5 upto the extent of 20% and 60%, respectively, as observed on 45 days

of inoculation, but they did not differentiate into any zoosporangium at this pH level (Table IIA, Graph IV). Thus vegetative cells of *R. hieroglyphicum* could survive pH 3.5 three times more than those of *C. glomerata*, while those of *P. oedogonia* and of all blue-green algae studied could not survive pH 3.5 at all. The survivability of vegetative cells in all algae as well as the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*, and that of zoosporangia in *R. hieroglyphicum*, all decreased progressively or inhibited altogether as the pH of the medium was lowered from 7.0 to 4.0 or enhanced from 8.0 to 11.0 (Table IIA, Graph IV). The pH values of both 7.0 as well as 8.0 were found to be almost equally favouring the survivability of vegetative cells as well as the differentiation of reproductive cells in all algae studied. The sexual phases in *Closterium acerosum* (Vidyavati & Nizam, 1973) and *Chlamydomonas geitleri* (Karel-Tetik and Sulek, 1987) were also favoured at pH 7.0 and 7.5, respectively.

Extreme pH levels of 4.0 / 5.0 or 10.0 / 11.0 not only decreased the formation of akinetes or zoosporangium but also their viability (Table IIA). Thus the survivability of algal vegetative cells to extremes of pH is directly linked with the differentiation of reproductive cells.

Although, in *C. glomerata,* about 60 and 40% vegetative cells survived pH 5.0 and 4.0, respectively on 45 days of inoculation, none of them differentiated into zoosporangia at these pH values. However, more than 80-90% vegetative cells of *C. glomerata* could survive and about 10-35% of them could form zoosporangia between pH values of 6.0 to 11.0 as observed on 45 days of inoculation (Table IIA, Graph IV). Thus

C. glomerata could survive alkaline side of pH somewhat better than other algae tested.

Among green algae studied, *P. oedogonia* was found to be most sensitive alga to extremes of pH of 3.5 and 11.0, while *R. hieroglyphicum*, the least (more particularly to acidic side); and this might probably be due to differences in their cell wall thickness or size of cells. *P. oedogonia* vegetative cells are slightly larger and have thin cell wall than of *R. hieroglyphicum.*

Among blue green algae used, *A. iyengarii* was the most sensitive alga to extremes of pH, while *W. prolifica* and *N. lobatus* were more or less equally most tolerant (Table II A, Graph IV) and this might be due to much delicate filaments of the former than of the latters. The formation of zoosporangium in *C. glomerata* and *R. hieroglyphicum* seems to be comparatively more sensitive to extremes of pH than was akinete formation in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia.*

Akinete or zoospore germination

No akinete of *A. iyengarii, W. prolifica, N. lobatus* or *P. oedogonia* or that of zoospore of *C. glomerata* or *R. hieroglyphicum* was found to germinate at pH 3.5 or 4.0 (Table II B, Graph IV), (akinetes or zoosporangia formed at pH 4.0 were also found to be not viable at all, Table II A). The percentage germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus* or *P. oedogonia* or that of zoospores in *C. glomerata* or *R. hieroglyphicum* decreased progressively or inhibited altogether as

the pH of culture medium decreased from 7.0 to 5.0 or increased from 8.0 to 11.0 (Table II B, Graph IV). The zoospore germination in *Stigeoclonium pascheri* also decreased at pH 6.0 and 10.0 and was completely absent at pH 5.0 (Agrawal, 1985).

Like survivability of vegetative cells or differentiation of reproductive cells from vegetative cells, germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus, P. oedogonia* as well as of zoospores in *C. glomerata* and *R. hieroglyphicum* also occurred maximally at pH 7.0 as well as 8.0 (Table IV, Graph IV). The viability of akinetes or zoosporangia harvested from pH 7.0 or 8.0 was maximum, while that of those harvested from extremes of pH was severely reduced (Table IV, Graph IV).

Mason (1965) observed that in *Cladophora* sp., pH of pond water during akinete germination was 8.1 to 8.3. The optimum pH for akinete germination of *Anabaena cylindrica* (Yamamoto, 1976) and *Anabaena vaginicola* (Rai and Pandey, 1981) were 7-8 and 7-9, respectively. In *Stigeoclonium pascheri*, akinete or zoospore germination also occurred maximally at pH 8.0 (Agrawal, 1984, 1985).

Thus the survivability of vegetative cells, the formation of akinetes or zoosporangia and germination of akinetes or zoospores in present algae, all were maximum at pH 7.0 and 8.0. pH extremes decreased the survivability of vegetative cells, formation of akinetes or zoosporangia, their viability, and germination of akinetes and zoospores in present algae.

Table II A : Percentage formation of dead cells (D) in all algae, akinetes (A) in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and zoosporangium (Z) in *C. glomerata* and *R. hieroglyphicum* at different pH values of the culture medium[a]

D, A, Z	Days of inoculation	pH							
		3.5	4.0	5.0	6.0	7.0	8.0	10.0	11.0
					A. iyengarii				
D	30	100	65	43	0	0	0	2	5
	60	-	80	65	25	3	5	25	50
A	30	0	0	0	0	25	10	0	0
	60	0	20[d]	35[d]	55[c]	86	84	50[c]	30[d]
					W. prolifica				
D	30	90	60	55	2	0	0.5	4	11
	60	100	75	70	5	2	3	10	15
A	30	0	20	25	35	20	46	40	30
	60	0	25[d]	30[c]	42[b]	83	75	64[c]	50[d]
					N. lobatus				
D	30	60	50	8	0	0	0	0	0
	60	100	80	25	10	3	5	30	40
A	30	0	30	28	32	45	45	30	20
	60	0	20[d]	50[d]	64[c]	84	80	50[c]	30[d]

					P. oedogonia				
D	15	100	75	42	10	1	8	10	20
	45	-	85	76	66	7	5	80	90
A	15	0	0	0	15	10	18	0	0
	45	0	15[d]	24[d]	34[c]	45	44	20[c]	10[d]
					C. glomerata				
D	15	2	2	1	0	3	0	0	0
	45	80	60	40	6	4	7	8	13
Z	15	0	0	0	0	10	0	10	15
	45	0	0	0	10[b]	35	30	30[c]	25[d]
					R. hieroglyphicum				
D	15	20	10	2	0	2	0	0	15
	45	40	20	24	19	5	15	30	45
Z	15	0	0	0	0	13	13	0	0
	45	0	4[d]	10[d]	20[b]	40	40	15[c]	8[d]

[a]All values represent rounded means of three replicates.
[b]Viability 10-20% as compared to those formed at pH 7.0.
[c]Viability 5-10% as compared to those formed at pH 7.0.
[d]No viability at all.

Table II B : Percentage germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus, P. oedogonia* and zoospores in *C. glomerata, R. hieroglyphicum* at different pH on 15 days of inoculation[a]

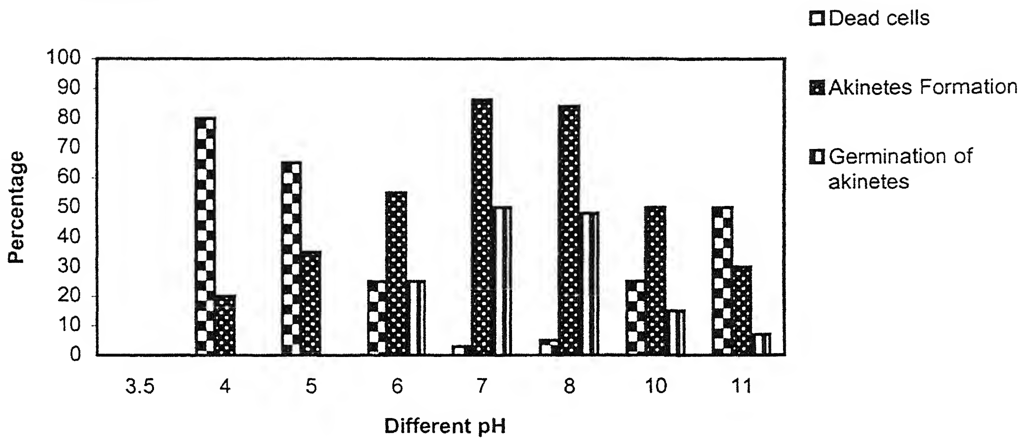
Algae	pH							
	3.5	4.0	5.0	6.0	7.0	8.0	10.0	11.0
A. iyengarii	0	0	0	25	50	48	15	7
W. prolifica	0	0	2	21	60	55	10	6
N. lobatus	0	0	5	32	65	62	25	15
P. oedogonia	0	0	0	35	70	67	5	2
C. glomerata	0	0	0	30	50	48	7	0
R. hieroglyphicum	0	0	10	28	55	53	12	6

[a] All values represent rounded means of three replicates.

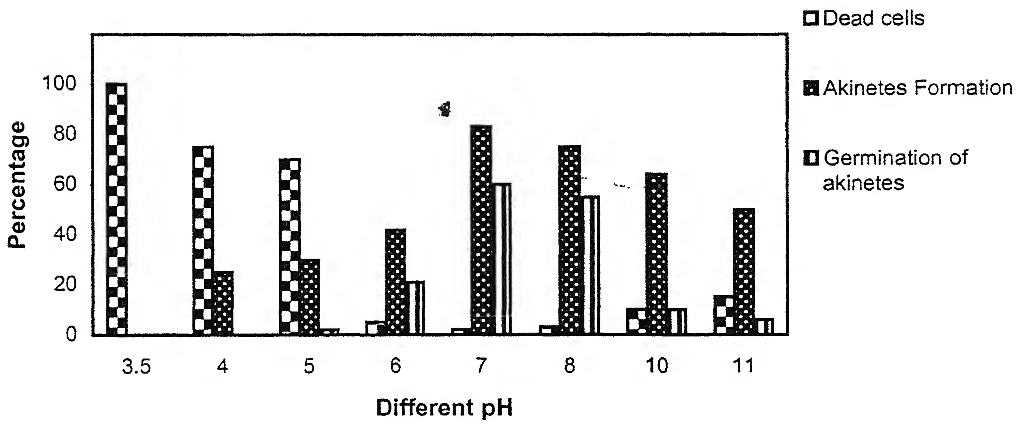
GRAPH- IV

THE PERCENTAGE FORMATION OF DEAD CELLS FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES AND ZOOSPORES IN ALGAE STUDIED AT DIFFERENT pH LEVELS

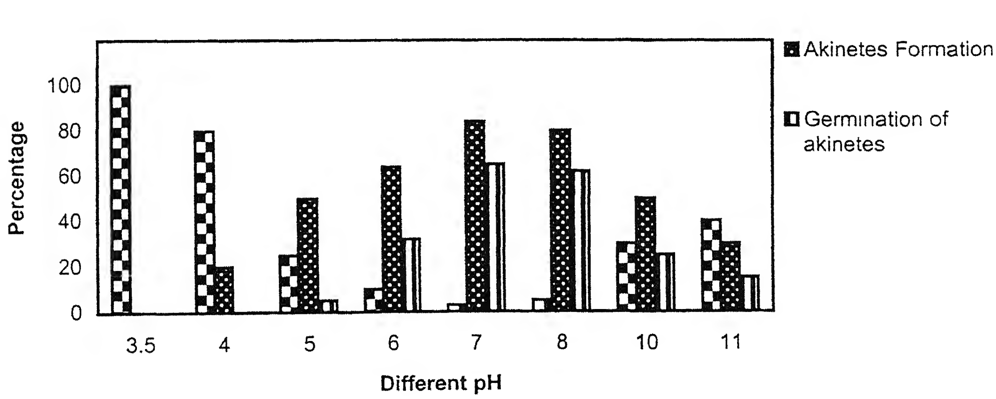
A. iyengarii



W. prolifica

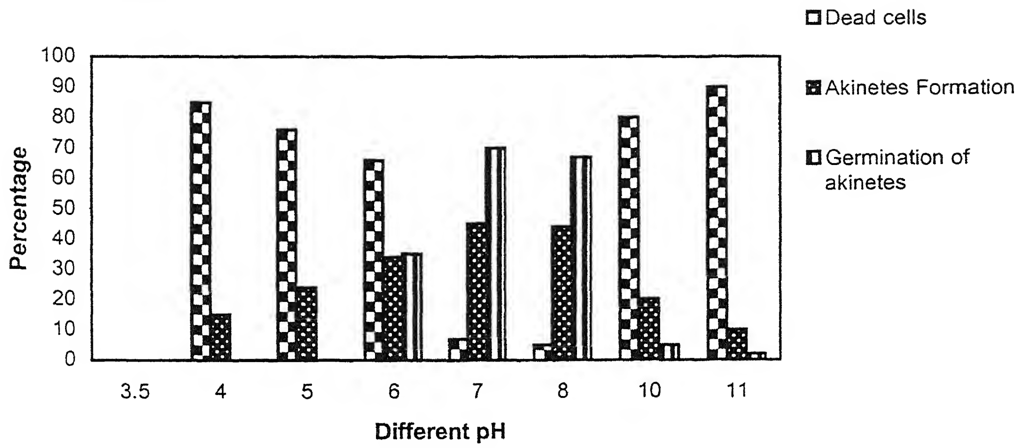


N. lobatus

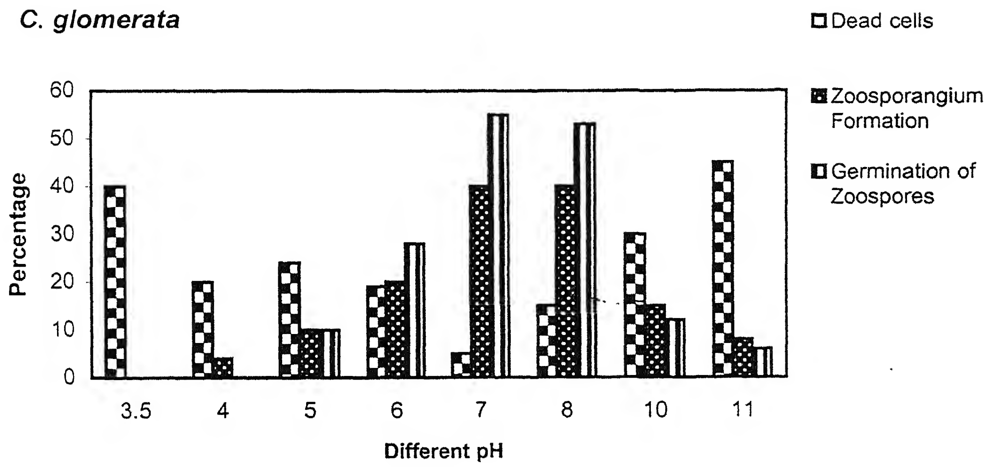


Contd.

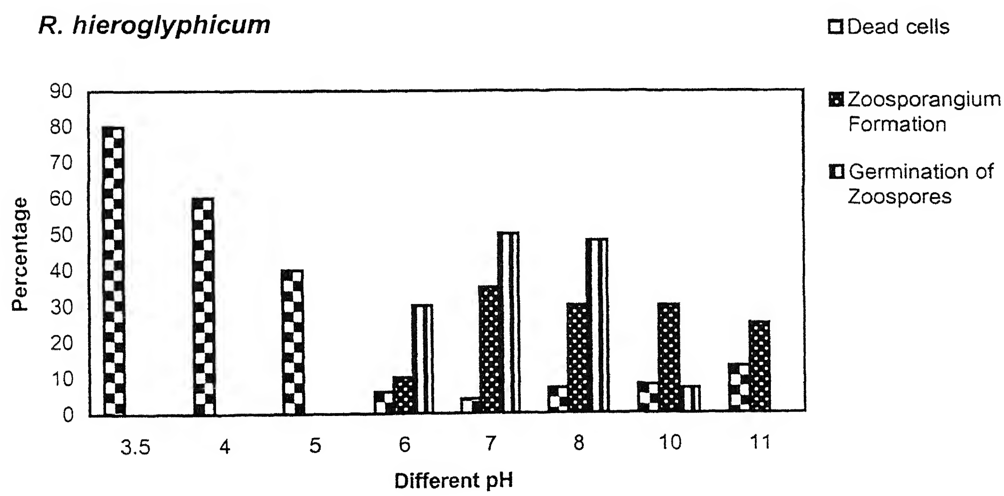
P. oedogonia



C. glomerata



R. hieroglyphicum



CHAPTER V

EFFECTS OF PESTICIDES ON THE SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN DIFFERENT ALGAE USED

CHAPTER V

EFFECTS OF PESTICIDES ON THE SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN DIFFERENT ALGAE USED

Various kinds of synthetic organic compounds are used as pesticides in order to protect agricultural crops against pest. Studies relating to the effects of pesticides on algae are mainly concerned with the fate of nitrogen-fixing blue-green algae in paddy fields (Venkataraman, 1972; Sharma, 1986; Mishra *et al.*, 1989).

It was observed by Hirang *et al.* (1955) that pesticide parathion at 1 to 5 p.p.m. did not affect the growth of blue-green alga *Tolypothrix tenuis* but killed herbivores in the agricultural field. Parathion at 1 p.p.m. also did not affect the growth in *Anacystis nidulans*, but DDT at the same level adversely affected the growth of the same alga (Gregory *et al.*, 1969).

Venkataraman and Rajyalakshmi (1972) observed that dithane at 5 p.p.m. was lethal to some strains of *Anabaena* spp. and *Nostoc* spp. but *Anabaena viriabilis* could tolerate even 500 p.p.m. of 2,4-D. Singh (1974) observed that *Cylindrospermum* sp. could tolerate without any effect 150 p.p.m. of 2,4-D in liquid cultures.

Kar and Singh (1979) observed that 4 p.p.m. of Gammexane was algistatic to *Nostoc muscorum* as well as to *Wollea bharadwajae.*

Megharaj *et al.* (1988) observed that Carbofuran applications to soil at the levels of 0.5-1 kg/ha either did not affect or slightly enhanced the growth of blue-green algal population. It was observed by Dikshit and Tiwari (1990) that Bavistin at 100 and 400 p.p.m. proved lethal to *Aulosira fertilissima* and *Scytonema stuposum*, respectively. Interaction of blue-green algae with pesticides have been reviewed by Adhikary (1998).

The present work was undertaken to see the effects of varying concentrations (1 to 50 p.p.m.) of different pesticides like insecticide-carbofuran (3% Carbofuran + 97% carrier and auxillaries, Pesticide India Ltd.), and phorate (90% phorate, Pesticide India Ltd.); herbicide - 2,4-D (80%, 2,4-Dichlorophenoxy-acetic acid + 20% other ingredients, Tropical Agro); and fungicides-dithane (Mancozeb Tech 88.23%, Bayer India Ltd.) and bavistin (50% Carbendazin HAL) on the survivability of vegetative cells in all algae used; formation and germination of akinetes in *Anabaena iyengarii, Westiellopsis prolifica, Nostochopsis lobatus* and *Pithophora oedogonia*; and on the formation of zoosporangia and germination of zoospores in *Cladophora glomerata* and *Rhizoclonium hieroglyphicum.* Viability of akinetes or zoosporangia formed in presence of different pesticides was also determined.

METHODS

The graded amounts of different pesticides used were mixed separately with the culture medium so as to prepare the solutions of desired concentrations. The concentrations of different pesticides used

range between 1 to 50 p.p.m.. The pH of the pesticide containing media was adjusted to 7.5 prior to autoclaving.

Survivability of vegetative cells and formation of akinetes and zoosporangia

In order to observe the effects of different pesticides on the survivability of vegetative cells, and on the formation of akinetes or zoosporangia in different algae used, the seven-day-old, actively growing vegetative filaments of all algae were used as a source of inoculum. Controls were maintained in standard BG 11 medium. All inoculated tubes were placed in the culture chamber and were examined on 60 days of inoculation so as to determine the percentage of dead cells, if any, in all algae used; that of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*; and that of zoosporangia in *C. glomerata* and *R. hieroglyphicum* with respect to total number of about 5000-6000 vegetative cells counted.

Viability of akinetes or zoosporangia formed

The akinetes or zoosporangia harvested from different concentrations of pesticides after 60 and 45 days of inoculation, respectively, were washed with distilled water and inoculated in to standard BG 11 medium. The percentage germination of akinetes or the percentage of empty zoosporangia which have released zoospores, out of total akinetes or zoosporangia inoculated was estimated on 15 day of inoculation.

Germination of akinetes or zoospores

Mature akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*, and freshly released zoospores of *C. glomerata* (adhering to coverslips) and *R. hieroglyphicum* (adhering to parent filaments), formed in standard BG 11 medium, were separately inoculated into culture tubes containing different concentrations of various pesticides used. Controls were maintained in standard BG 11 medium. All inoculated culture tubes were placed in culture chamber under controlled culture conditions.

The percentage germination of akinetes or that of zoospores was determined by counting about 3000 akinetes or zoospores on 15 days after inoculation.

RESULTS AND DISCUSSION

(i) Survivability of Vegetative cells, formation of reproductive structures and their viability

Even 1 p.p.m. of any of five pesticides used, viz. Carbofuran, 2,4-D, Dithane, Phorate or Bavistin decreased the survivability of *A. iyengarii* vegetative cells by about 80 to 95%, while 5 p.p.m. of any of them killed the alga entirely without formation of any akinete within 60 days of inoculation (Table III, Graph V to IX). Viability of akinetes formed at 1 p.p.m. of any of pesticides used was also very much reduced.

Venkataraman and Rajyalakshmi (1972) observed that Dithane at 5 p.p.m. proved lethal to some strains of *Anabaena* sp. and *Nostoc* sp. However, Sardeshpande and Goyal (1982) observed that 1 p.p.m. of

Phorate stimulated the growth of *Anabaena* sp. and *Calothrix* sp. Rath and Adhikary (1995) observed that Carbofuran at 0.01 to 0.5 μg/ml concentrations decreased the growth and nitrogen fixation of *Anabaena fertilissima* and *Anabaena viriabilis*.

W. prolifica and *N. lobatus* were found to be somewhat equally more tolerant blue-green algae to different pesticides used than was *A. iyengarii* (Table III, Graph V to IX), since they could survive and differentiate into akinetes (to some extent) even at 50 p.p.m. of Carbofuran, 2,4-D, Dithane, Phorate or Bavistin (Table III, Graph V to IX). Rath and Adhikary (1994) examined the relative tolerance of different blue-green algae viz. *Anabaena, Nostoc, Cylindrospermum, Calothrix, Scytonema, Aulosira* and *Westiellopsis* to Furadan and found that ensheathed forms were comparatively more tolerant to pesticides than unsheathed forms and thus could be more ideal for use as biofertilizers.

Although, akinete formation in *W. prolifica* and *N. lobatus* was observed to occur at all concentrations of pesticides used, it was very low as compared to controls. The viability of akinetes formed in presence of pesticides also decreased very much as compared to control akinetes (Table III).

In the present study, about 80-95% vegetative cells of *P. oedogonia* died without forming any akinete even at 1 p.p.m. of different pesticides used (Table III, Graph V to IX). Thus like blue green alga *A. iyengarii*, it was also a pesticide sensitive alga.

It is to be noted that *C. glomerata* vegetative cells were not tolerating at all even 1 p.p.m. of Dithane or 10 p.p.m. of Phorate, while *R. hieroglyphicum* vegetative cells could tolerate (upto about 50%) even 50 p.p.m. of Dithane or 10 p.p.m. of Phorate as reported on 60 days of inoculation (Table III, Graph VII, VIII). However, both *C. glomerata* and *R. hieroglyphicum* could tolerate (upto the extent of 45-65%) even 50 p.p.m. of Carbofuran, 2,4-D or Bavistin (Table III, Graph V, VI, IX).

Although, vegetative cells of *C. glomerata* survived to some extent the 5 p.p.m. of Phorate, or 10 p.p.m. of Carbofuran or Bavistin and those of *R. hieroglyphicum* the 1 p.p.m. of Phorate or 10 p.p.m. of Bavistin, they did not differentiate into zoosporangia at these levels of pesticides. Zoosporangia of *C. glomerata* and *R. hieroglyphicum* did not liberate any zoospore at any concentration of pesticides used. In both algae, no zoosporangia dehisce or zoospores germinate even at 1 p.p.m. of any of pesticides used (Table III).

Thus, zoosporangium dehiscence is much sensitive process than of its formation. The zoosporangia of both algae formed in presence of different pesticides did not dehesce when placed in control medium (Table III).

Akinete or zoospore germination :

No akinete germination in *A. iyengarii* was observed at 5 p.p.m. of all the pesticides used, while, akinetes of *W. prolifica* could germinate to some extent at 5 p.p.m. of Bavistin and 1 p.p.m. of rest of other pesticides.

Akinetes of *N. lobatus* could germinate to some extent at 1 p.p.m. of 2,4-D, Phorate and Bavistin, at 5 p.p.m. of Carbofuran and at 10 p.p.m. of Dithan (Table III, Graph V to IX). In *P. oedogonia*, akinete germination was observed only at 1 p.p.m. any of pesticides used. No zoospore of *C. glomerata* and *R. hieroglyphicum* germinated even at 1 p.p.m. of any of pesticides used.

Thus all pesticides used in the present study decreased at various levels or inhibited altogether the survivability of vegetative cells, the formation of akinetes or zoosporangia, their viability, and the germination of akinetes or zoospores in algae studied. Dehiscence of zoosporangia and zoospore germination both were much sensitive to pesticides than was akinete germination.

Table III : Percentage formation of dead cells (D) in all algae, formation of akinetes (A), and germination of akinetes (AG) in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*, and Zoosporangia formation (Z) and germination of zoospores (ZG) in *C. glomerata* and *R. hieroglyphicum* at different concentrations of pesticides used. Formation of dead cells, akinetes and zoosporangia were counted on 60 days of inoculation of vegetative filaments while akinetes or zoospore germination on 15 days after inoculation[a]

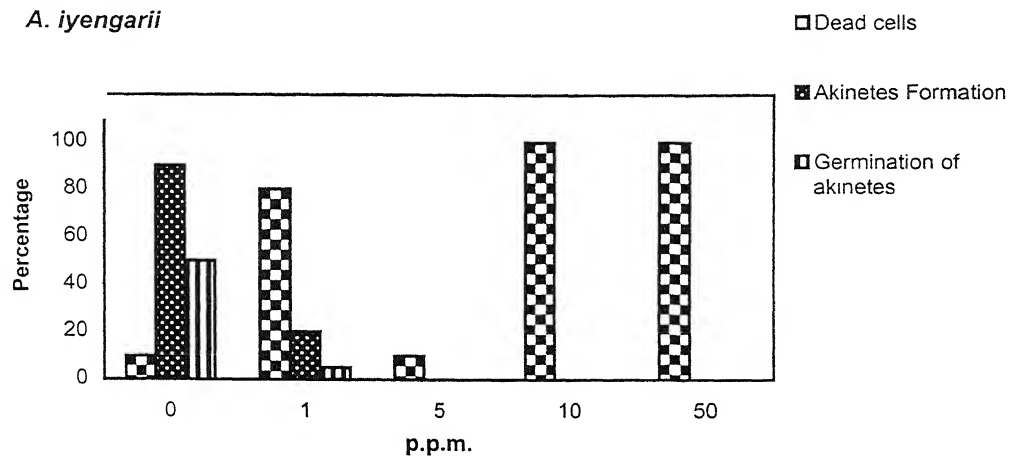
Pesticides (in ppm)		Algae																	
		A. iyengarii			*W. prolifica*			*N. lobatus*			*P. oedogonia*			*C. glomerata*			*R. hieroglyphicum*		
		D	A	AG	D	A	AG	D	A	AG	D	A	AG	D	Z	ZG	D	Z	ZG
Control		10	90	50	2	80	55	3	79	60	7	40	70	3	35	50	5	45	50
Carbo-furan	1	80	20^{c}	5	20	34^{b}	45	15	25^{d}	20	95	5^{d}	10	35	20^{d}	0	30	14^{d}	0
	5	100	0	0	80	20^{b}	0	65	13^{d}	2	96	4^{d}	0	40	13^{d}	0	25	5^{d}	0
	10	100	0	0	75	25^{c}	0	70	9^{c}	0	97	3^{d}	0	40	0	0	25	3^{d}	0
	50	100	0	0	70	10^{d}	0	90	5^{b}	0	98	2^{d}	0	55	0	0	35	2^{d}	0
2,4-D	1	95	5^{c}	0	40	10^{b}	5	30	15^{b}	10	95	5^{d}	12	10	10^{d}	0	20	10^{d}	0
	5	100	0	0	90	10^{b}	0	40	10^{b}	0	97	3^{d}	0	20	7^{d}	0	20	8^{d}	0
	10	100	0	0	95	5^{c}	0	55	15^{c}	0	98	2^{d}	0	30	5^{d}	0	30	7^{d}	0
	50	100	0	0	98	2^{d}	0	85	10^{d}	0	100	0	0	50	0	0	40	6	0
Dithane	1	90	10^{c}	0	10	32^{b}	3	20	35^{b}	20	90	10^{d}	15	100	0	0	25	12^{d}	0
	5	100	0	0	20	15^{b}	0	25	25^{b}	10	90	10^{d}	0	100	0	0	20	7^{d}	0
	10	100	0	0	25	30^{c}	0	40	12^{c}	4	95	5^{d}	0	100	0	0	45	5^{d}	0
	50	100	0	0	30	29^{d}	0	52	10^{d}	0	98	2^{d}	0	100	0	0	50	2^{d}	0

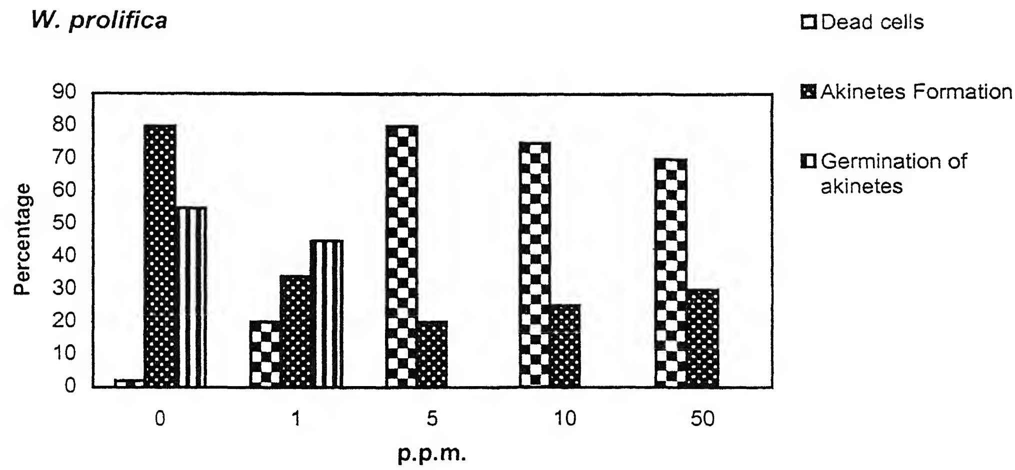
Phorate	1	85	15[c]	15	25	40[b]	25	40	30[b]	5	85	15[d]	8	35	12[d]	0	45	0	0
	5	100	0	0	35	30[b]	0	55	28[b]	0	98	2[d]	0	60	0	0	50	0	0
	10	100	0	0	55	22[c]	0	60	30[c]	0	98	2[d]	0	100	0	0	70	0	0
	50	100	0	0	60	11[d]	0	80	20[d]	0	100	0	0	100	0	0	100	0	0
Bavis-tin	1	95	5[d]	0	5	40[b]	20	10	30[b]	12	80	20[d]	10	15	20[d]	0	8	15[d]	0
	5	100	0	0	7	20[b]	5	10	25[b]	0	88	12[d]	0	25	7[d]	0	15	8[d]	0
	10	100	0	0	15	30[c]	0	20	20[c]	0	95	5[d]	0	35	0	0	40	0	0
	50	100	0	0	90	10[d]	0	85	15[d]	0	100	0	0	55	0	0	50	0	0

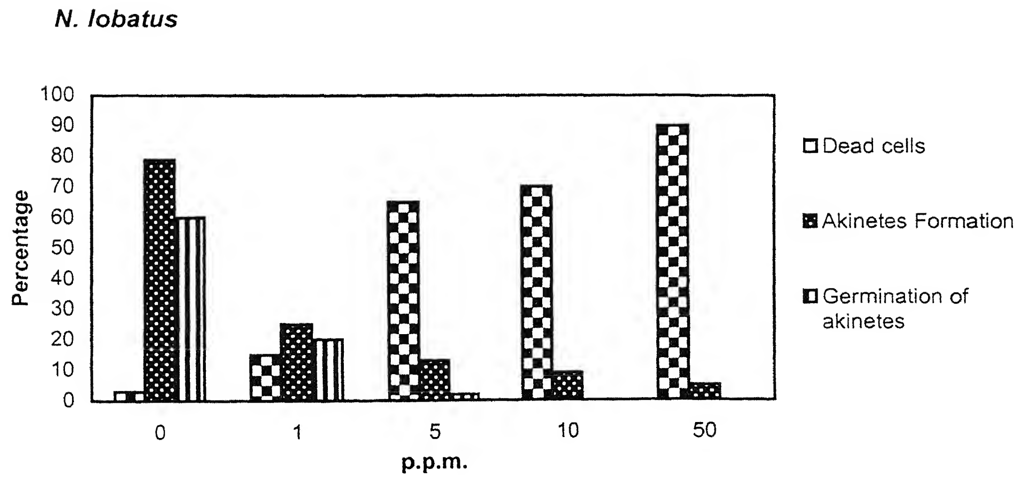
[a]Data represent rounded mean value of three replicates.
[b]Viability 10-20% as compared to those formed in controls.
[c]Viability 2-5% as compared to those formed in controls.
[d]No viability at all.

GRAPH- V

EFFECT OF CARBOFURAN ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES AND ZOOSPORES IN ALGAE STUDIED

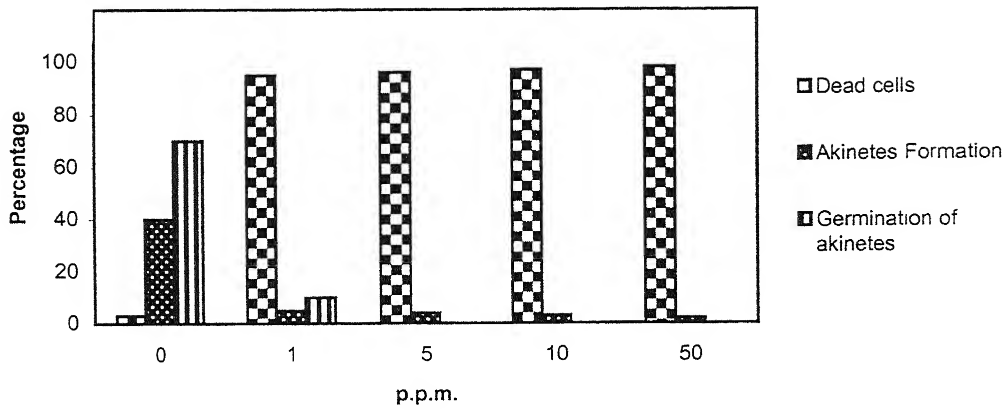




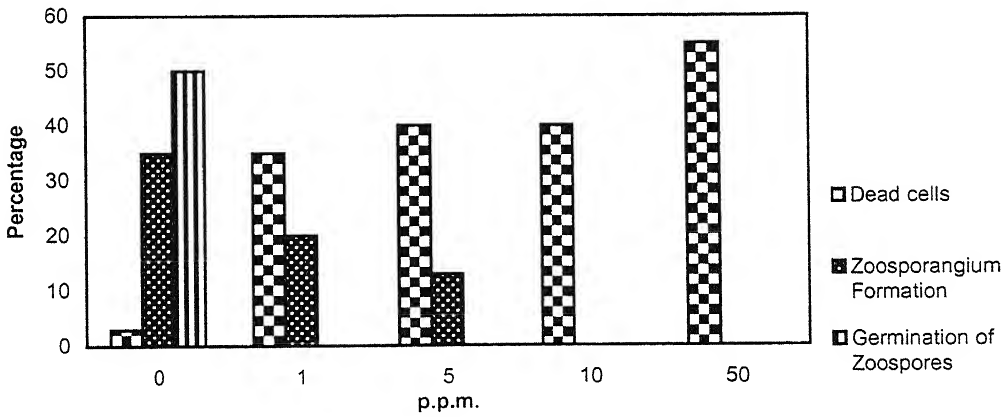


Contd.

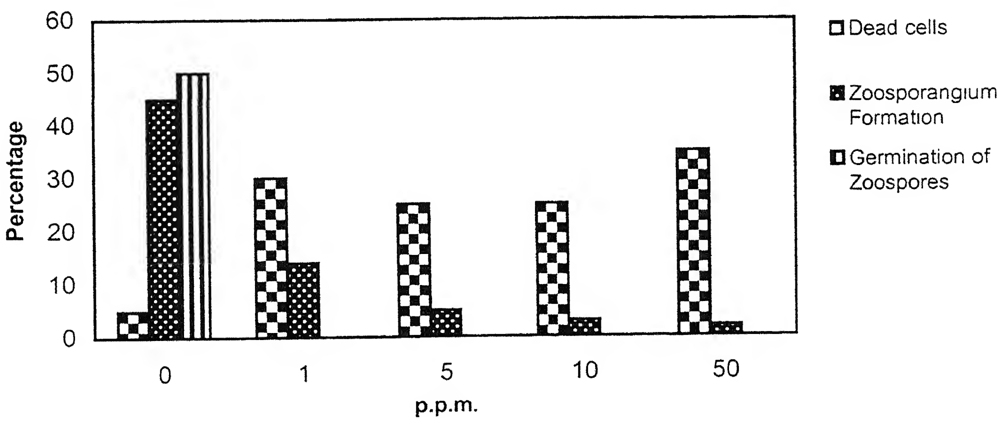
P. oedogonia



C. glomerata

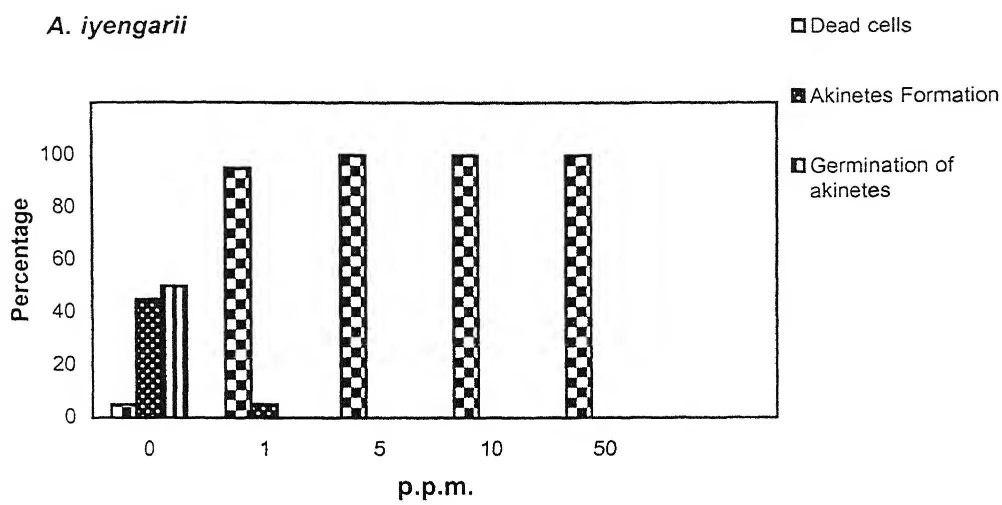


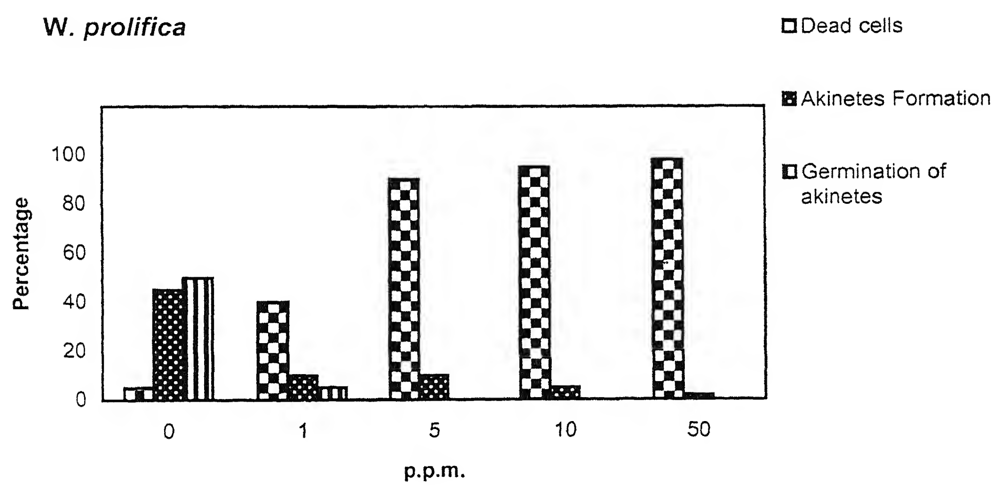
R. hieroglyphicum

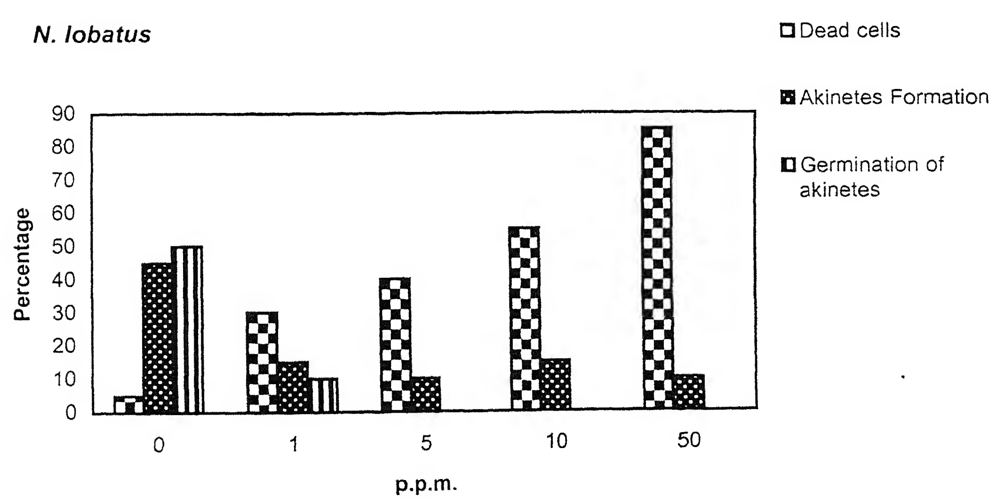


GRAPH- VI

EFFECT OF 2, 4-D ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATIN OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

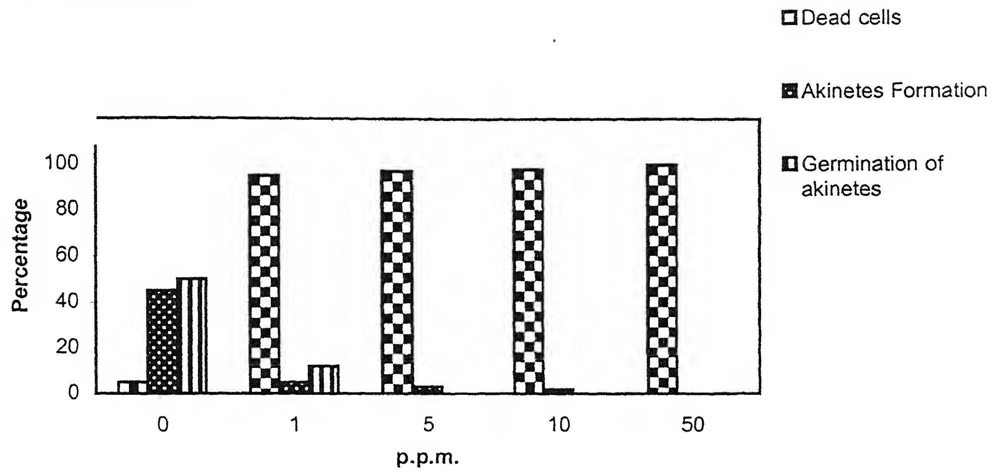




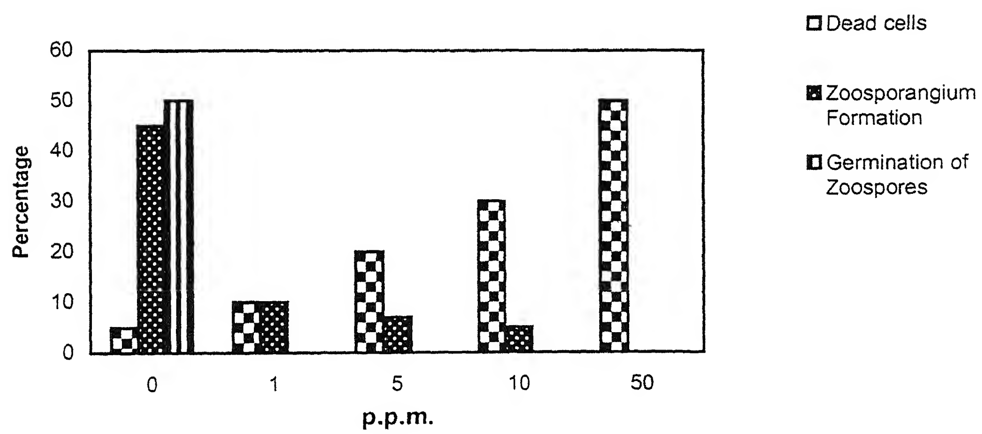


Contd.

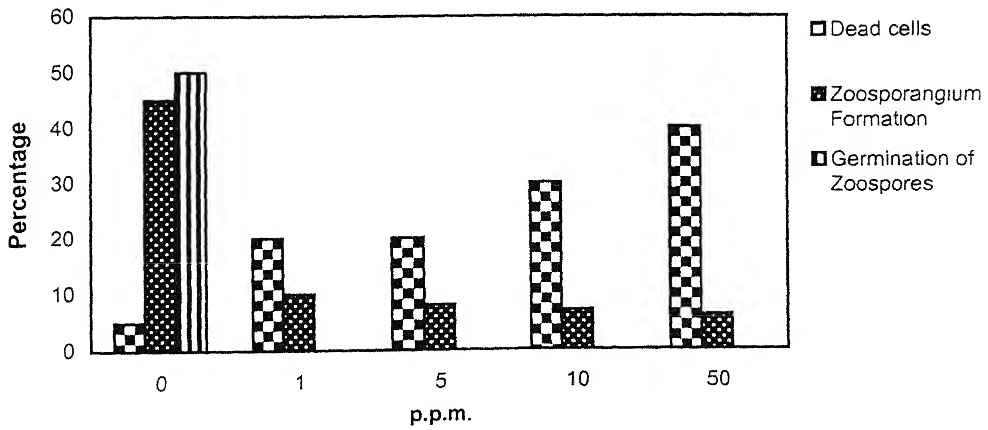
P. oedogonia



C. glomerata



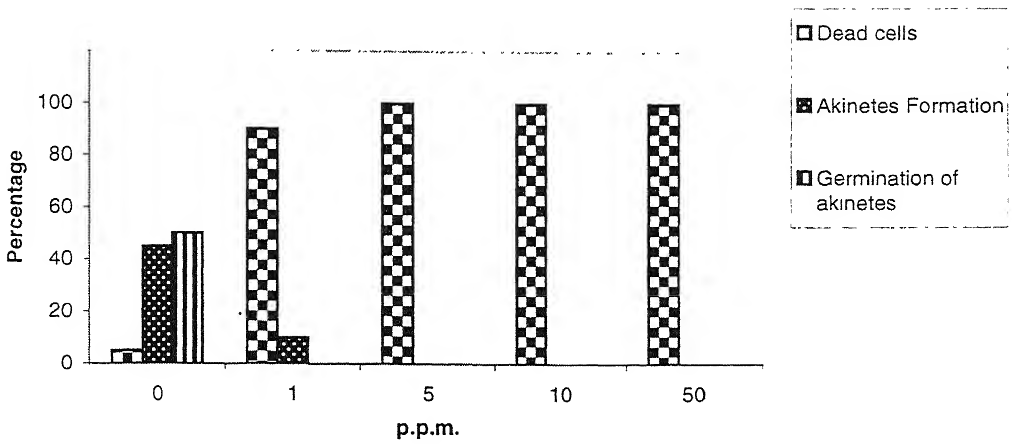
R. hieroglyphicum



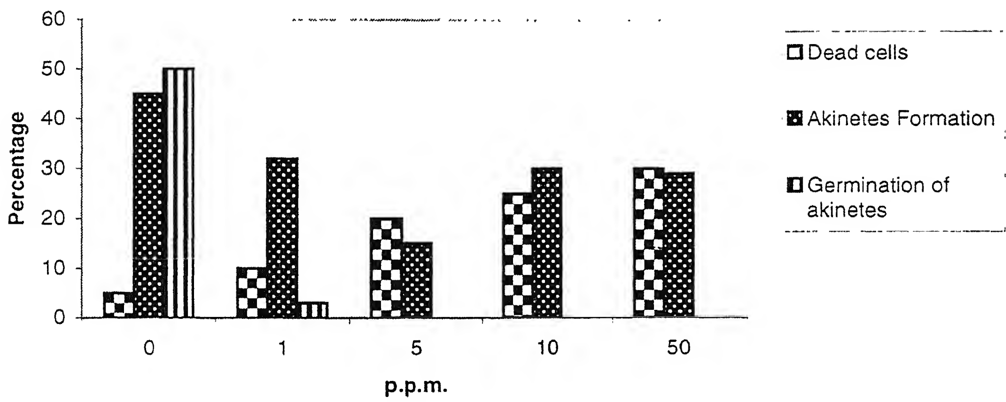
GRAPH- VII

EFFECTS OF DITHANE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

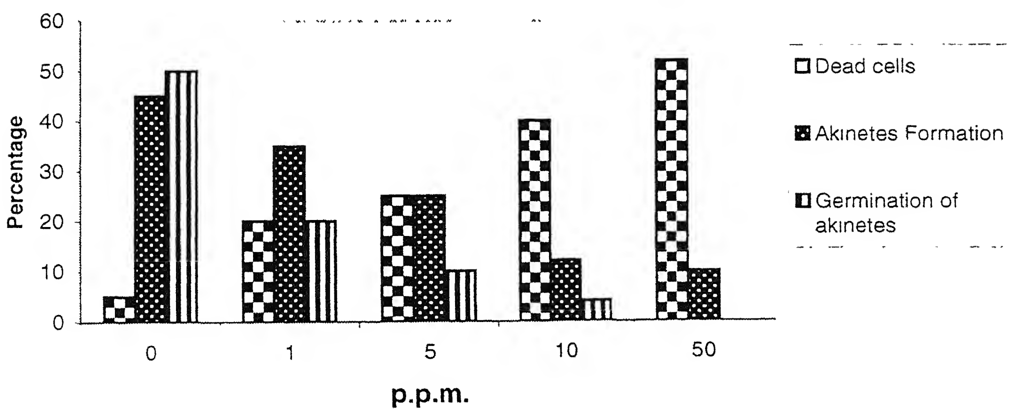
A. iyengarii



W. prolifica

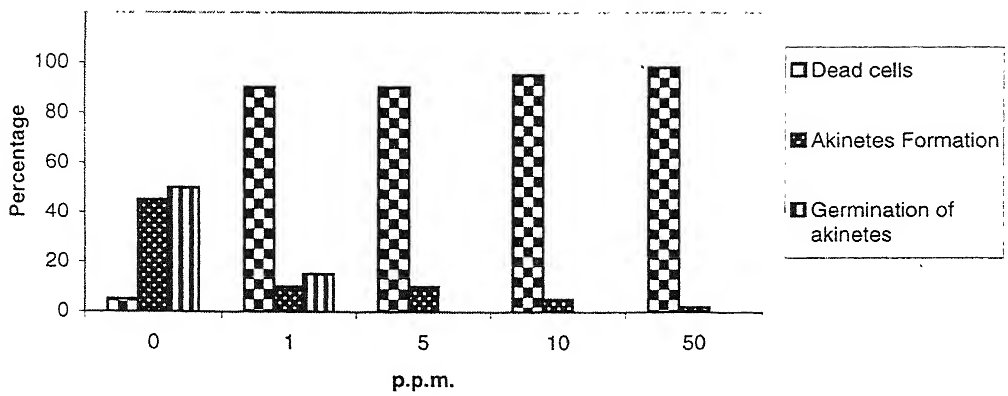


N. lobatus

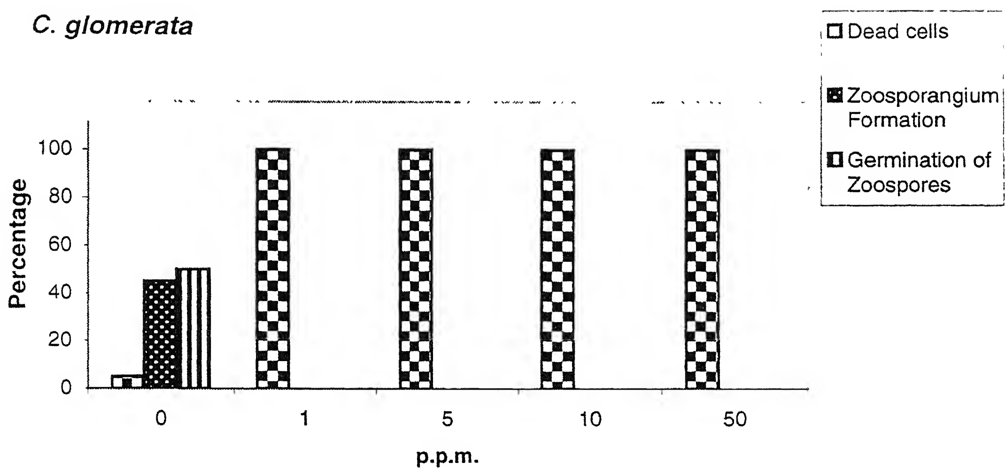


Contd.

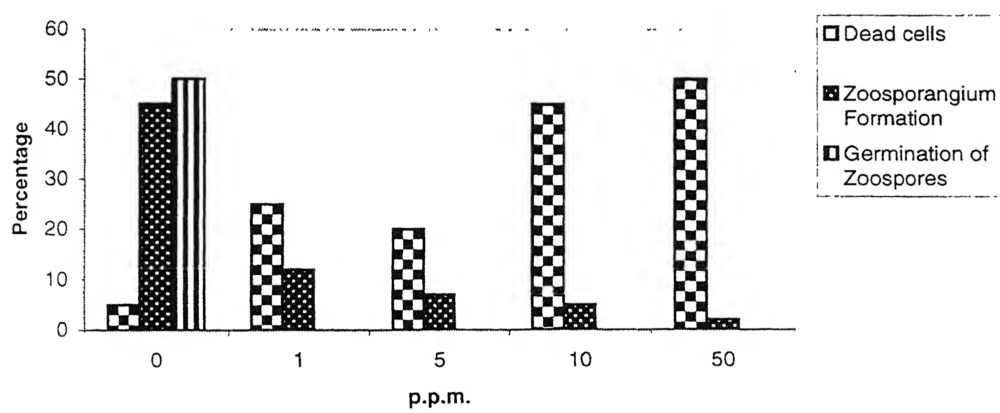
P. oedogonia



C. glomerata



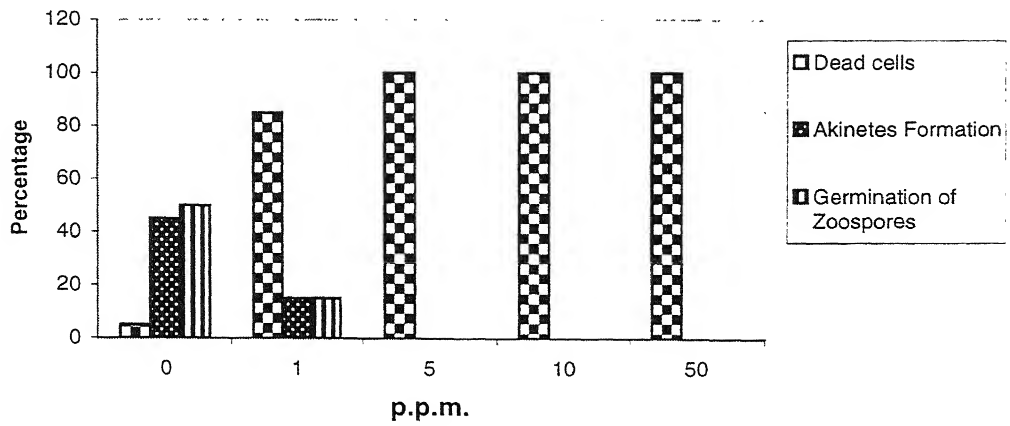
R. hieroglyphicum



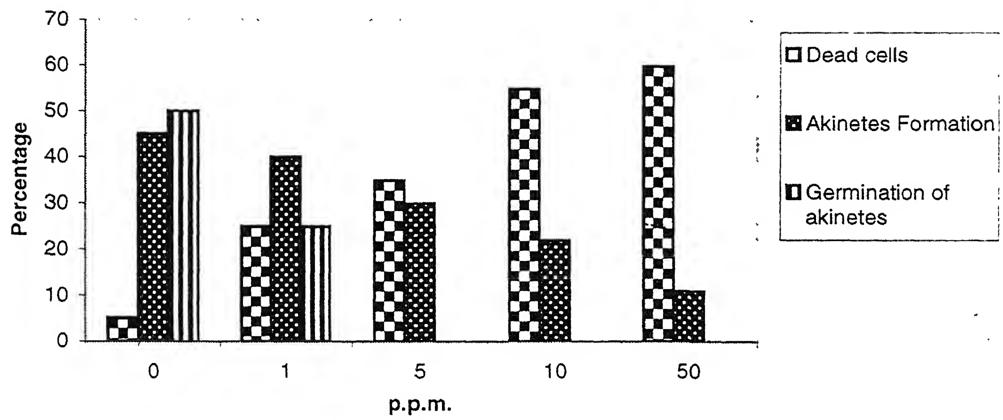
GRAPH- VIII

EFFECTS OF PHORATE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOSSPORES IN ALGAE STUDIED

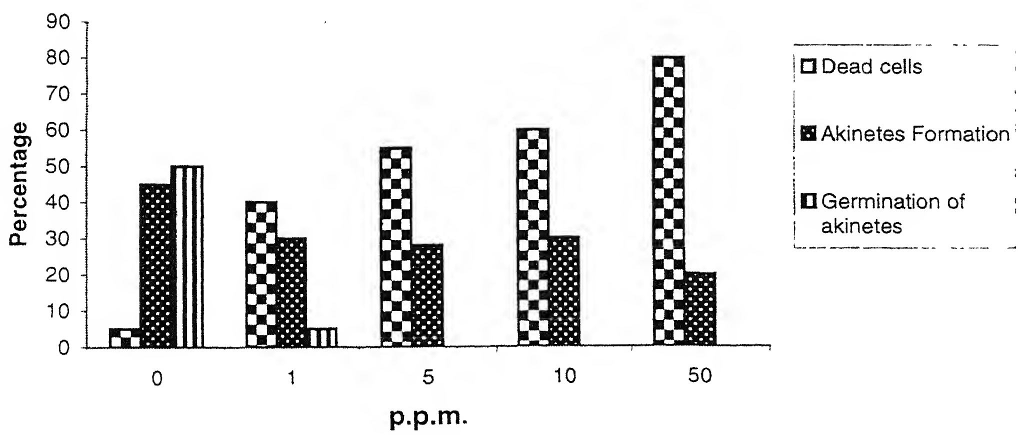
A. iyengarii



W. Prolifica

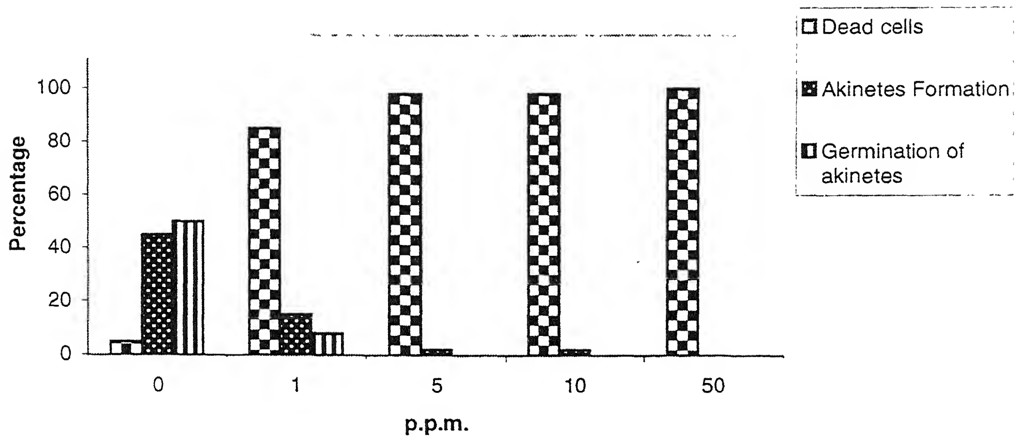


N. lobatus

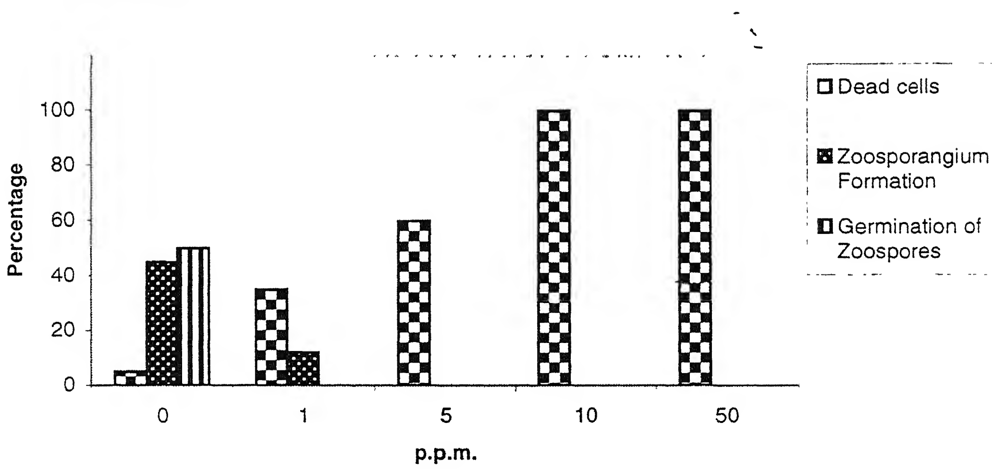


Contd.

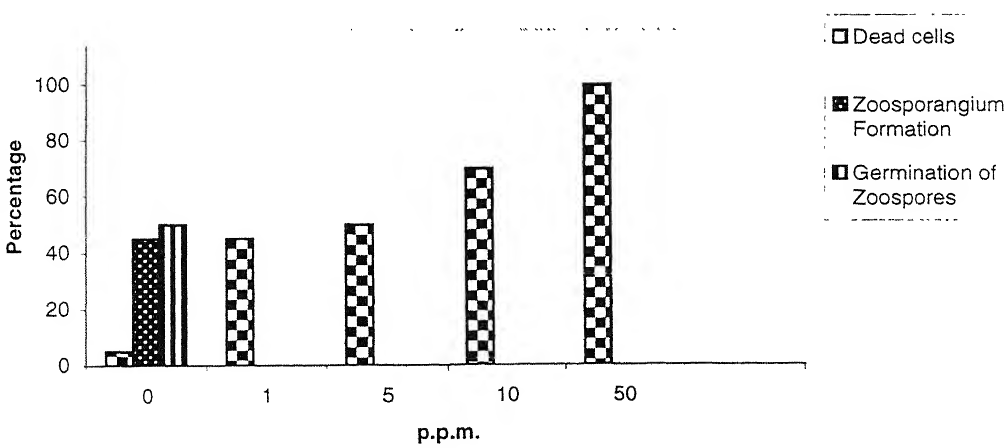
P. oedogonia



C. glomerata



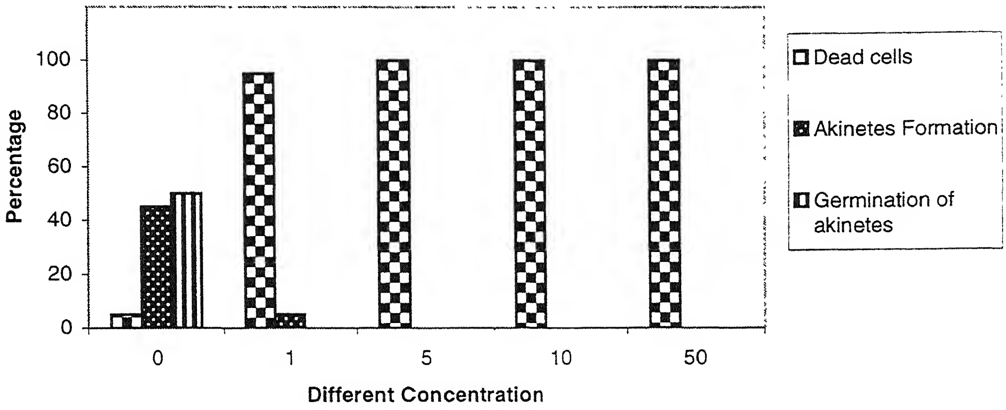
R. hieroglyphicum



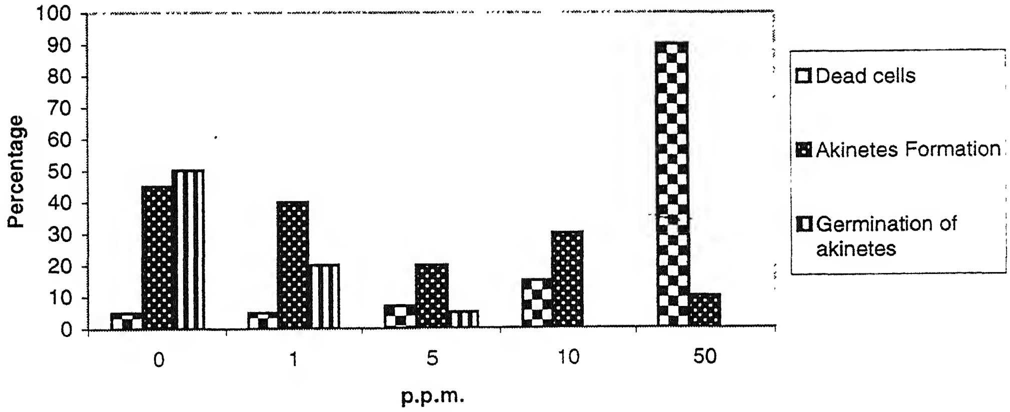
GRAPH- IX

EFFECTS OF BAVISTIN ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

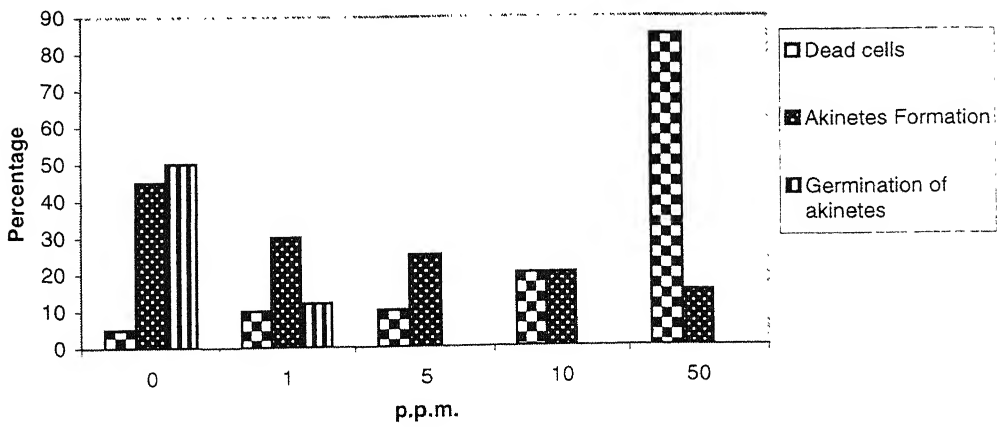
A. iyengarii



W. prolifica

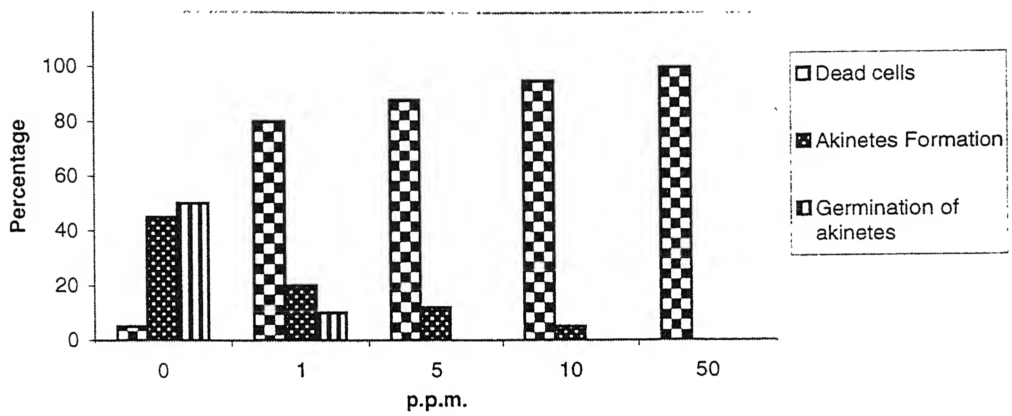


N. lobatus

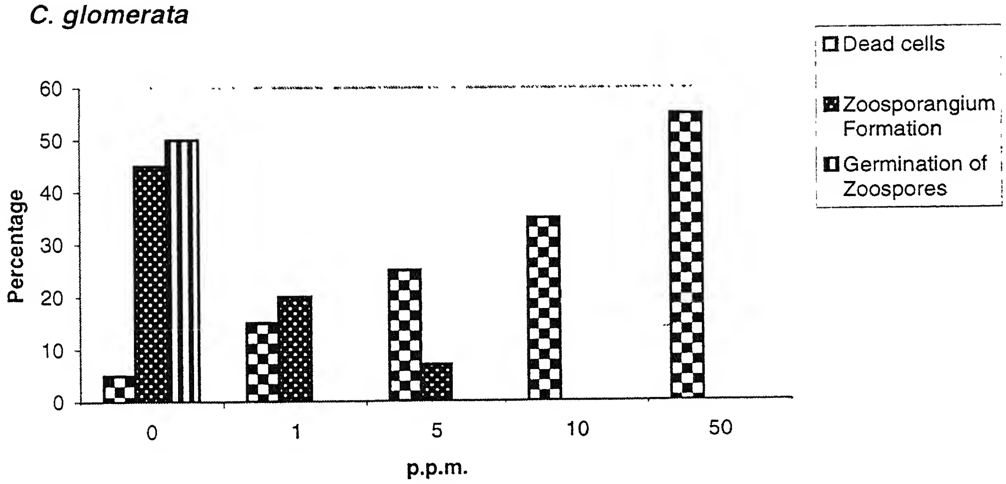


Contd.

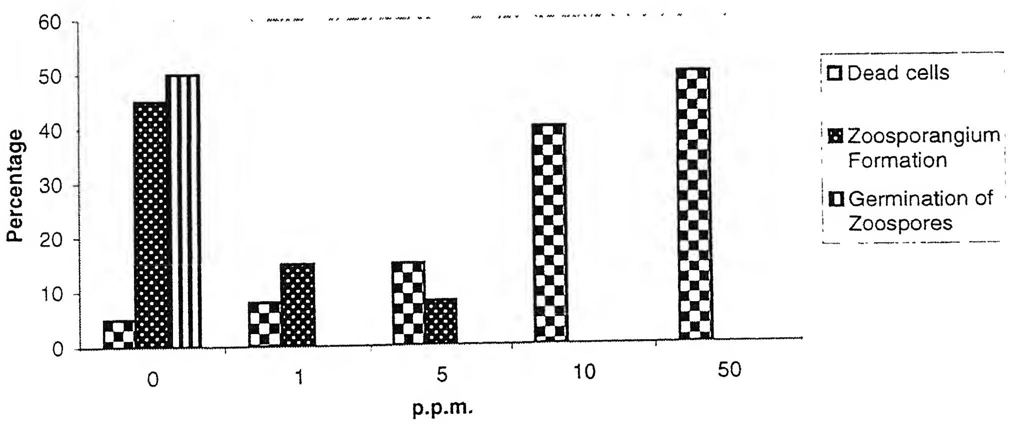
P. oedogonia



C. glomerata



R. hieroglyphicum



CHAPTER VI

EFFECTS OF HEAVY METALS ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

CHAPTER VI

EFFECTS OF HEAVY METALS ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

Some of the heavy metals are essential for growth and other metabolic processes in algae because they form co-factors and act as activators of different enzymes (Van Assche and Clijsters, 1990). Copper act as an essential component of plastocyanin (Katoh *et al.*, 1961), photosystem I (Bishop, 1964) and enzyme amine oxidase (Palenik and Morel, 1991); while zinc, as a prosthetic group for enzymes alkaline phosphatase, carbonic anhydrase and lactic dehydrogenase (Price, 1962; Coleman, 1984; Cembella *et al.*, 1984). Zinc also play an important role in the activity of mRNA (Altman *et al.*, 1968), maintenance of integrity of ribosomes (Rai *et al.*, 1981a), and transport and assimilation of nutrients (Rueter and Morel, 1981).

Cobalt in the form of vitamin B_{12} is required to increase the dry weight and nitrogen content of marine algae *Ulva, Dictyota* and *Pterocladia* (Nasr and Bakheet, 1970). Manganese play an important role in synthesis of glycolate and galactosyl glyceride and for activity of photosystem II (Tanner *et al.*, 1960; Constantopoulos, 1970; Price and Morel, 1994), and is thus required for algal growth (Rai *et al.*, 1981a).

Heavy metals are essential for various metabolic processes only in trace quantities, and they become toxic to living beings when present in high concentrations. Toxicity of heavy metals to algae have been reviewed by Whitton (1984), Eichenberger (1986) and De Filippis and Pallaghy (1994). Although, manganese was needed for growth and photosynthesis in algae (Eyster *et al.*, 1958), negative relationship exist between manganese and sexuality in diatom *Ditylum brightwellii* (Steele, 1965). Copper at certain levels has been found to inhibit functioning of photosynthetic pigments in several algae (Gross *et al.*, 1970; Gachter *et al.*, 1973; Thomas and Seibert, 1977; Mallick and Rai, 1990; Rai *et al.*, 1991).

Copper, nickel, lead and iron has been found to interfere with nutrients uptake in *Anabaena doliolum* (Rai and Dubey, 1989). Copper and lead interfer enzyme nitrogenase activity in *Nostoc muscorum* (Rai and Raizada, 1989). Copper also produced reduction in pyrenoid number in *Dunaliella minuta* (Visviki and Rachlin, 1992).

Inhibition of photosystem I reaction centre following exposure to mercury was reported in *Anacystis nidulans* (Kimimura and Katoh, 1972; Kojima *et al.*, 1987). Toxic potential of mercury depends on its multiple binding capacity with electron transport chain (Rai *et al.*, 1991). Mercury and zinc depress nitrate reductase activity in *Chlorella vulgaris* (Rai *et al.*, 1991); while chromium, nickel, lead and silver in *Nostoc muscorum* (Rai *et al.*, 1990).

The present study was undertaken to see the effects of varying concentrations of 0.5 to 50 p.p.m. of different heavy metals like copper sulphate (99% $CuSO_4 .7H_2O$, E. Merck, India Ltd.), zinc oxide (99% ZnO, Sarabhai Chemicals, Baroda), Mercuric chloride (98% $HgCl_2$, SD Fine company, India), lead nitrate (99% $PbNO_3$, SD Fine Company, India), and cobalt nitrate (97% $Co(NO_3)_2$, E. Merck, India Ltd.) on the survivability of vegetative cells in all algae used; formation and germination of akinetes in *Anabaena iyengarii, Westiellopsis prolifica, Nostochopsis lobatus* and *Pithophora oedogonia*; formation of zoosporangia and germination of zoospores in *Cladophora glomerata* and *Rhizoclonium hieroglyphicum*; and on the viability of akinetes and zoosporangia formed.

METHODS

The graded amounts of different heavy metals used were mixed with standard BG 11 medium prior to autoclaving, pH adjusted to 7.5, so as to prepare the solutions of desired concentrations of 0.5 to 50 p.p.m..

(i) Survivability of vegetative cells and formation of akinetes and zoosporangia

In order to observe the effects of different heavy metals on the survivability of vegetative cells, and on the formation of akinetes and zoosporangia in different algae used, the seven-day-old actively growing vegetative filaments of all algae were used as a source of inoculum. Controls were maintained in standard BG 11 medium. All inoculated culture tubes were placed in the culture chamber and were examined on

60 days of inoculation so as to determine the percentages of dead cells, if any, in all algae used; that of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*; and that of zoosporangia in *C. glomerata* and *R. hieroglyphicum* with respect to total number of about 5000-6000 vegetative cells counted.

(ii) Viability of akinetes or zoosporangia formed

In order to determine the viability of akinetes or zoosporangia formed at different levels of heavy metals, akinetes and zoosporangia were harvested on 60 and 45 days after inoculation, respectively, washed with distilled water and inoculated into basal media and placed in culture chamber. Akinetes or zoosporangia bearing filaments harvested from basal media, inoculated similarly served as controls. The percentage germination of akinetes or the percentage of empty zoosporangia which have released zoospores, out of total of akinetes or zoosporangia inoculated was estimated on 15 day of inoculation.

(iii) Germination of akinetes or zoospores

Mature akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*; and freshly released zoospores of *C. glomerata* (attached to coverslips) and of *R. hieroglyphicum* (adhering to parent filaments), formed in standard BG 11 medium, were, separately, inoculated into culture tubes containing different concentrations of various heavy metals used. Controls were maintained in standard BG 11 medium. All inoculated culture tubes were placed in culture chamber under controlled culture conditions.

The percentage germination of akinetes or that of zoospores was determined by counting about 3000 akinetes or zoospores on 15 days after inoculation.

RESULT AND DISCUSSION

(i) Survivability of vegetative cells, formation of akinetes or zoosporangia and their viability

Survivability of *A. iyengarii* vegetative cells decreased to about 70-95% at 1 p.p.m. of any of different heavy metals used, while all vegetative cells of the alga died without forming any akinete at 5 p.p.m. of any of heavy metals used (Table IV, Graph X to XIV). Although, *A. iyengarii* formed akinetes to some extent at 1 p.p.m. of all heavy metals used, they were not viable at all (Table IV). However, akinetes of *A. iyengarii* formed at 0.5 p.p.m. of any of heavy metals used were viable by about 2-5% as compared to control akinetes (Table IV).

A reduction in growth following exposure to heavy metals could be due to inhibition of either normal cell division as reported for *Dunaliella tertiolecta* exposed to mercury (Davies, 1976), or for *Chlorella vulgaris* exposed to cadmium, copper and mercury (Rosko and Rachlin, 1977) or inhibition of nutrients uptake as reported for *Anabaena doliolum* exposed to chromium and lead (Mallick and Rai, 1990).

W. prolifica vegetative cells were quite tolerant to different heavy metals used and could survive even 10 p.p.m. of any of heavy metals used by about 50-85 percent. The alga also formed akinetes to some

extent even at 10 p.p.m. of any of heavy metals used, but no akinete was formed at 50 p.p.m. of any of heavy metals used. Akinetes of *W. prolifica* formed at 0.5 p.p.m. of any of five heavy metals used were viable by about 20-30%; while those formed at 1 p.p.m. by about 2-5%; and those formed at 5 p.p.m. of any of five heavy metals used not at all. (Table IV).

Upto about 15% vegetative cells of *N. lobatus* survived 10 p.p.m. of $CuSO_4$; while upto about 50 to 75%, 10 p.p.m. of each of zinc oxide, mercuric chloride, lead nitrate and cobalt nitrate as observed on 60 days of inoculation (Table IV, Graph X to XIV). The akinete formation in *N. lobatus* occurred upto 10 p.p.m. of any of all heavy metals used but did not occur at all and all algal cells died at 50 p.p.m. of any of heavy metals used. At 10 p.p.m. of cobalt nitrate, akinete formation occurred only upto 5 percent level (Table IV, Graph XIV). Akinetes of *N. lobatus* formed in presence of 0.5 p.p.m. of any of heavy metals used were viable by about 20-30%, while those formed at 1 p.p.m. of any of them by about 2-5%, and those formed at 5 p.p.m. of any of them not at all. (Table IV). Capasso and Pinto (1982) observed that green alga *Spermatozopsis acidophila* show high resistance to heavy metals, particularly to copper. This phenomenon might be interpreted as a consequence of evolutionary adaptation to highly acidic environments.

In the present study, *P. oedogonia* was the most sensitive alga to all heavy metals used, since 88-95% of its vegetative cells died without forming any akinete even at 1 p.p.m. of any of heavy metals used.

Akinetes of *P. oedogonia* formed at 0.5 p.p.m. of any of heavy metals used were viable by only about 2-5%; while those formed at 1 p.p.m. of any of heavy metals used not at all. (Table IV). Formation of aplanospores in *Haematococcus lacustris* (Xylander & Braune, 1994) and that of akinetes in *Pithophora oedogonia* (Chaudhary and Singh 1986) was inhibited in presence of nickel and mercuric chloride, respectively.

Vegetative cells of green algae *C. glomerata* and *R. hieroglyphicum* survived 10 p.p.m. of each of $CuSO_4$, $HgCl_2$ and $Co(NO_3)_2$ respectively by about 60 and 30%; 60 and 50%, and 5 and 32% but those of *C. glomerata* could not survive 10 p.p.m. of lead nitrate, and those of both algae, 10 p.p.m. of zinc oxide as observed on 60 days after inoculation. (Table IV, Graph X to XIV). *R. hieroglyphicum* vegetative cells could survive 10 p.p.m. of lead nitrate by about 45% on 60 days after inoculation. Differentiation of zoosporangia by both algae occur to a very reduced extent when grown in 0.5 p.p.m. of copper sulphate, zinc oxide and mercuric chloride and in 1 p.p.m. of lead nitrate and cobalt nitrate. The zoosporangia of *C. glomerata* and *R. hieroglyphicum* when formed in presence of any of heavy metals used were not viable since they did not liberate any zoospores. (Table IV). Toxic effects of copper are evident in *C. vulgaris* at even at very low concentration of 10^{-7} M (Greenfield, 1942). While, *Stigeoclonium tenue* was found growing abundantly in zinc polluted waters containing 20 p.p.m. of zinc (Harding and Whitton, 1976).

(iii) Akinete or zoospore germination

About 5-25% akinetes of *A. iyengarii* could germinate at 0.5 p.p.m. of either mercuric chloride, copper sulphate, zinc oxide, lead nitrate or cobalt nitrate as reported on 15 days after inoculation, but no akinete of *A. iyengarii* germinated at 1 p.p.m. of any of heavy metals used. (Table IV, Graph X to XIV).

About 2-5% akinetes of *W. prolifica* and *N. lobatus* germinated at 5 p.p.m. of copper sulphate, but none of their akinetes germinated at the same level of zinc oxide, mercuric chloride, lead nitrate or cobalt nitrate. (Table IV, Graph X to XIV).

In *P. oedogonia*, akinete germination was observed to occur to a very reduced extent of only 2-10% at 0.5 p.p.m. of copper sulphate, zinc oxide, lead nitrate and cobalt nitrate. Akinete germination in *P. oedogonia* was absent at 0.5 p.p.m. of mercuric chloride (Table IV, Graph X to XIV). Mercuric chloride also inhibited akinete germination in *Pithophora oedogonia* (Chaudhary and Singh, 1986). Agrawal and Sarma (1982b) reported that the presence of H_3BO_3, $ZnSO_4$, $MnCl_2$, MoO_3, $CuSO_4$ and $Co(NO_3)_2$ in basal medium served as a check in reaching maximum level of akinete germination in *Stigeoclonium pascheri.*

The zoospores of *C. glomerata* and *R. hieroglyphicum* germinated to a very reduced extent of 3 to 10% at 0.5 p.p.m. of copper sulphate, zinc oxide, lead nitrate and cobalt nitrate but did not germinate at all at 0.5

p.p.m. of mercuric chloride and at 1 p.p.m. of any of heavy metals used. (Table IV, Graph X to XIV).

Thus copper, zinc, mercury, lead and cobalt, all at 0.5 to 10 p.p.m. inhibited progressively or suppressed completely the survivability of vegetative cells, the formation of akinetes or zoosporangia, their viability and the germination of akinetes or zoospores in algae studied. The vegetative cells of *A. iyengarii* and *P. oedogonia* were much sensitive to all heavy metals used than were rest of other algae studied. Growth inhibition in algae following heavy metals exposure might be due to inhibition of functioning of photosynthetic pigments, enzymatic activities or nutrients uptake or damage to cell membrane (Stokes, 1983; De Filippis and Pallaghy, 1994).

Table IV : Percentage dead cells counts (D), formation (A) and germination (AG) of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and Zoosporangia formation (Z) and germination of zoospore (ZG) in *C. glomerata* and *R. hieroglyphicum* at different concentrations of heavy metals. Dead cells count and formation of akinetes and zoosporangia were reported after 60 days of inoculation of vegetative filament while akinetes or zoospores germination on 15 days after inoculation[a]

Conc. of heavy metals (in ppm)		*A. iyengarii*			*W. prolifica*			*N. lobatus*			*P. oedogonia*			*C. glomerata*			*R. hieroglyphicum*		
		D	A	AG	D	A	AG	D	A	AG	D	A	AG	D	Z	ZG	D	Z	ZG
Control	0	10	90	50	2	75	55	3	83	60	3	40	70	3	35	50	5	45	50
$CuSO_4$ $7H_2O$	0.5	10	35^{c}	25	10	70^{b}	30	5	70^{b}	20	77	23^{c}	10	10	10^{d}	10	25	20^{d}	5
	1	95	5^{d}	0	15	50^{c}	10	50	40^{c}	5	95	5^{d}	0	20	0	0	20	0	0
	5	100	0	0	25	30^{d}	5	75	25^{d}	2	100	0	0	30	0	0	60	0	0
	10	100	0	0	35	10^{d}	0	85	15^{d}	0	100	0	0	40	0	0	70	0	0
	50	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
ZnO	0.5	34	25^{c}	15	9	60^{b}	22	20	60^{b}	15	80	20^{c}	8	10	20^{d}	4	25	12^{d}	7
	1	80	5^{d}	0	10	50^{c}	6	20	50^{c}	2	90	10^{d}	0	30	0	0	40	0	0
	5	100	0	0	15	25^{d}	0	34	25^{d}	0	96	4^{d}	0	60	0	0	80	0	0
	10	100	0	0	25	10^{d}	0	50	10^{d}	0	100	0	0	100	0	0	100	0	0
	50	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
$HgCl_2$	0.5	7	40^{c}	5	10	70^{b}	10	13	72^{b}	8	85	15^{c}	0	10	2^{d}	0	19	7^{d}	0
	1	80	20^{d}	0	15	65^{c}	0	18	64^{c}	0	92	8^{d}	0	20	0	0	20	0	0
	5	100	0	0	25	30^{d}	0	27	30^{d}	0	97	0	0	25	0	0	27	0	0
	10	100	0	0	30	25^{d}	0	40	15^{d}	0	100	0	0	40	0	0	50	0	0
	50	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0

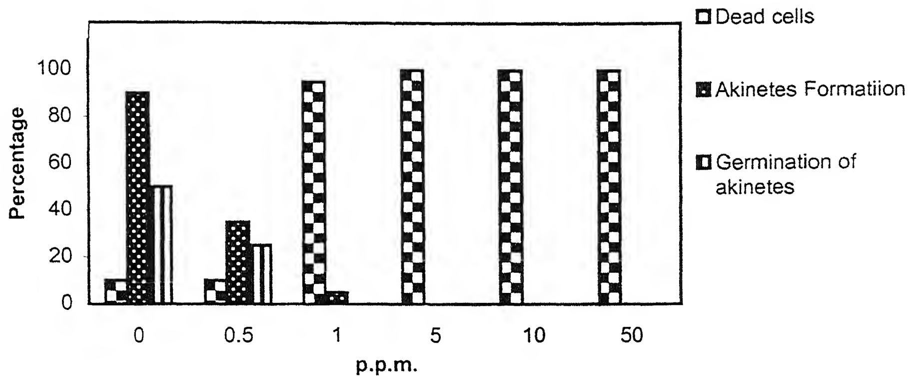
$PbNO_3$	0.5	10	40[c]	24	10	72[b]	15	8	73[b]	10	85	15[c]	5	18	13[c]	3	25	7[c]	4
	1	70	30[d]	0	13	60[c]	6	12	50[c]	4	88	12[d]	0	20	5[d]	0	40	8[d]	0
	5	100	0	0	15	52[d]	0	16	34[d]	0	90	10[d]	0	40	0	0	42	0	0
	10	100	0	0	20	33[d]	0	25	20[d]	0	95	5[d]	0	100	0	0	55	0	0
	50	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
$Co(NO_3)_2$	.5	30	35[c]	15	13	67[b]	20	7	72[b]	15	80	20[c]	2	12	16[c]	5	13	10[c]	4
	1	80	10[d]	0	27	50[c]	9	18	60[c]	6	90	10[d]	0	20	7[d]	0	28	4[d]	0
	5	100	0	0	40	30[d]	0	21	34[d]	0	100	0	0	40	0	0	40	0	0
	10	100	0	0	50	9[d]	0	30	5[d]	0	100	0	0	95	0	0	68	0	0
	50	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0

[a]All values represent rounded mean of three replicates
[b]Viability 20-30% as compared to those formed in standard BG 11 medium.
[c]Viability 2-5% as compared to those formed in standard BG 11 medium.
[d]No viability at all.

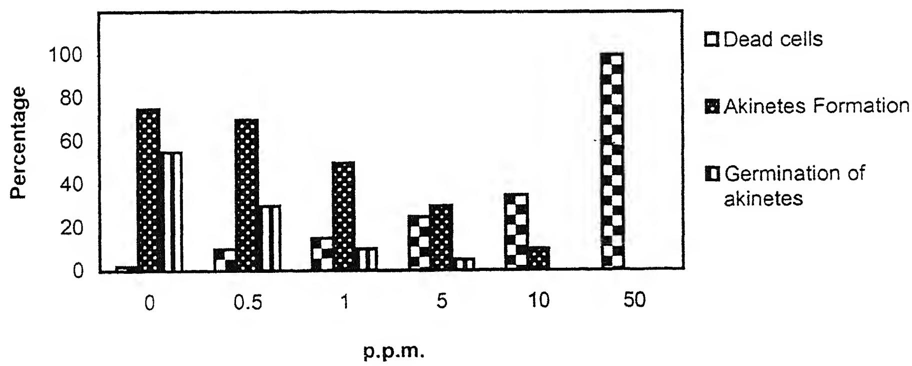
GRAPH- X

EFFECTS OF COPPER SULPHATE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

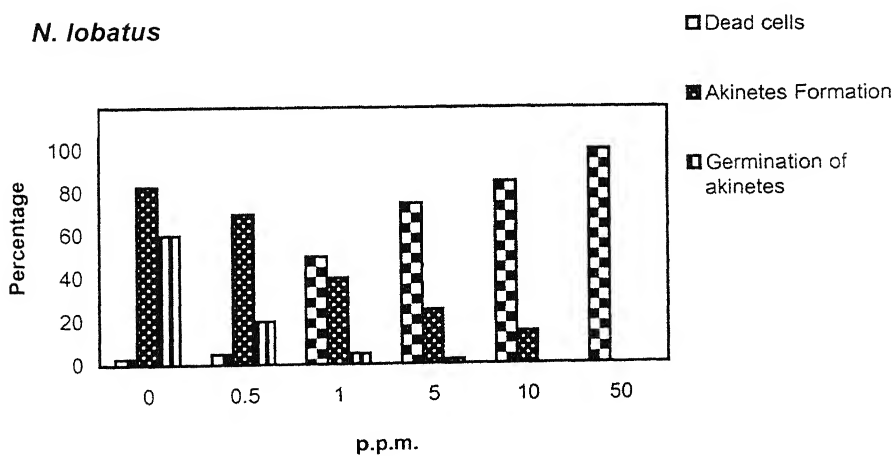
A. iyengarii



W. prolifica

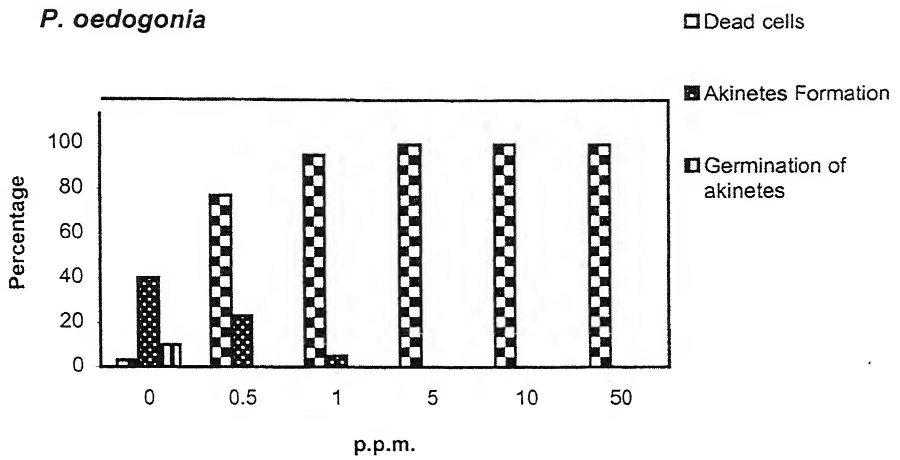


N. lobatus

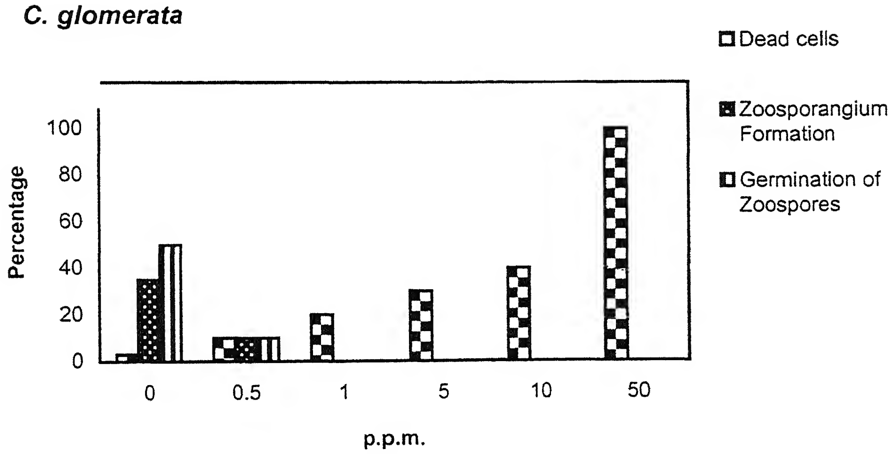


Contd.

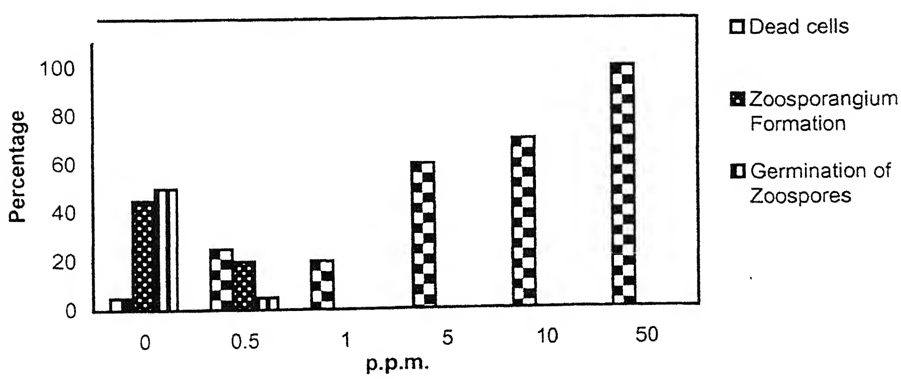
P. oedogonia



C. glomerata



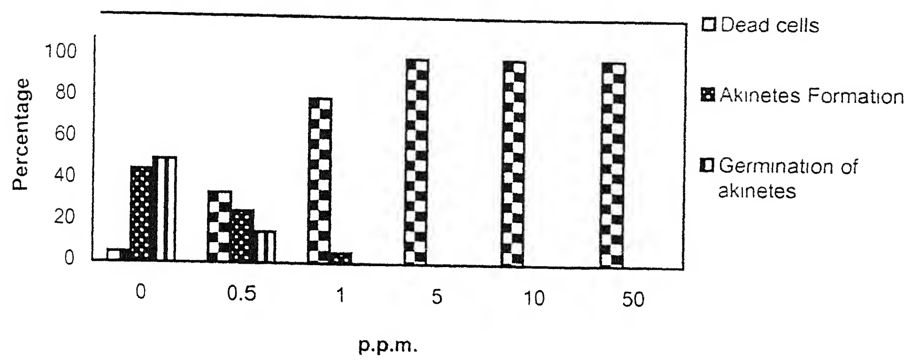
R. hieroglyphicum



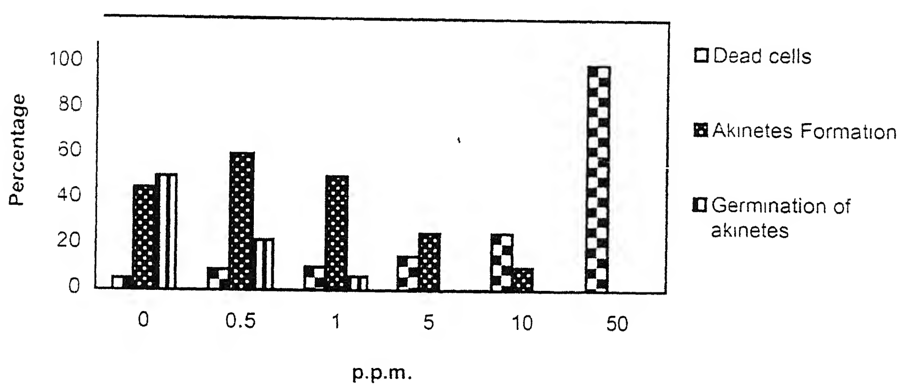
GRAPH- XI

EFFECTS OF ZINC OXIDE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

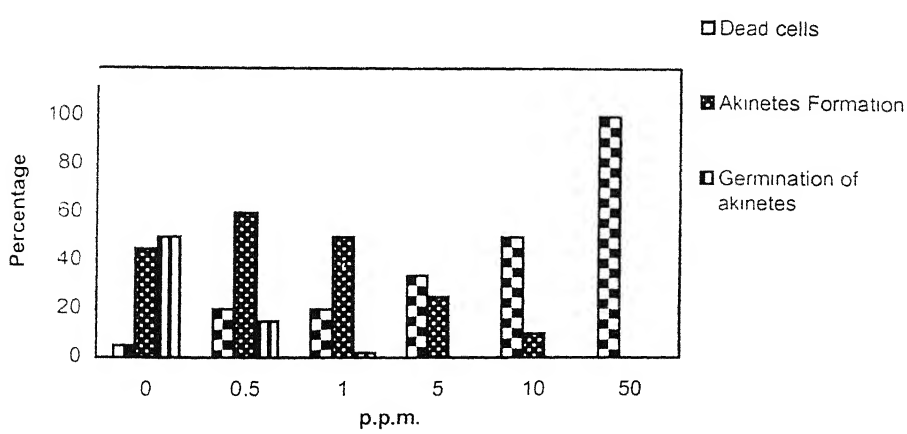
A. iyengarii



W. prolifica

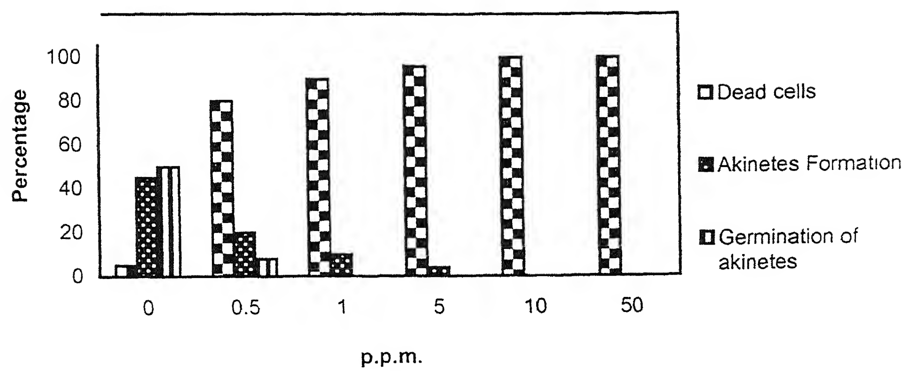


N. lobatus

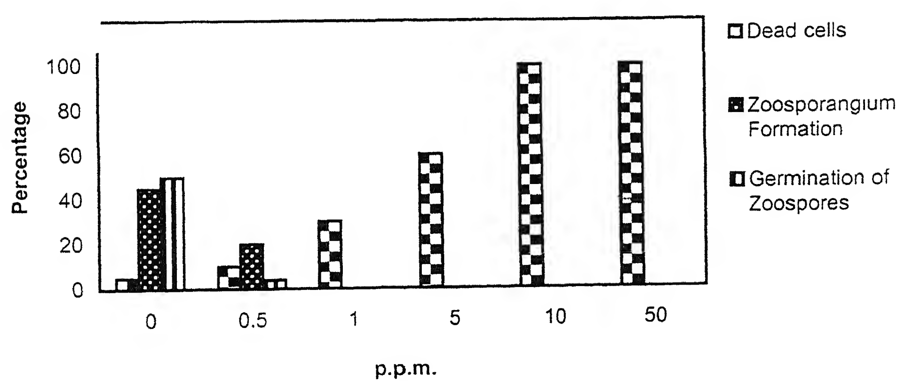


Contd.

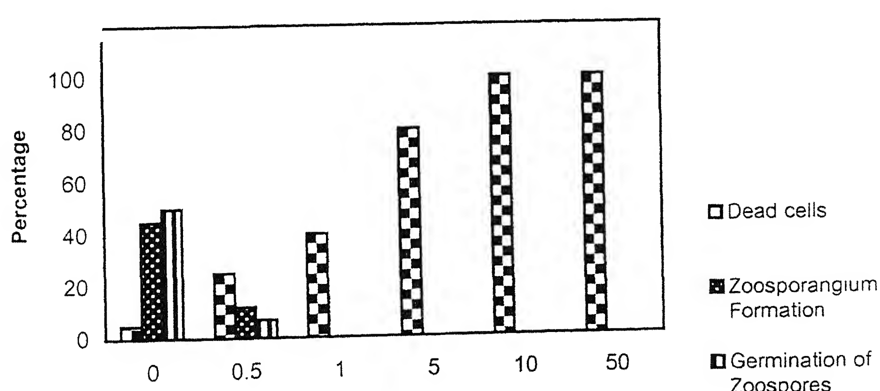
P. oedogonia



C. glomerata

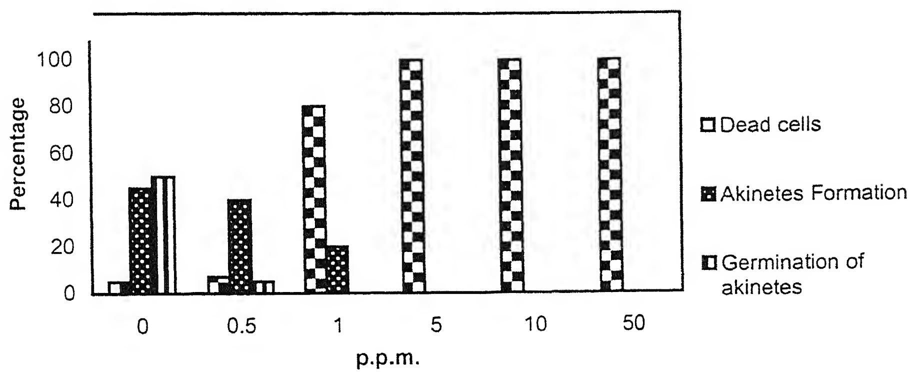


R. hieroglyphicum

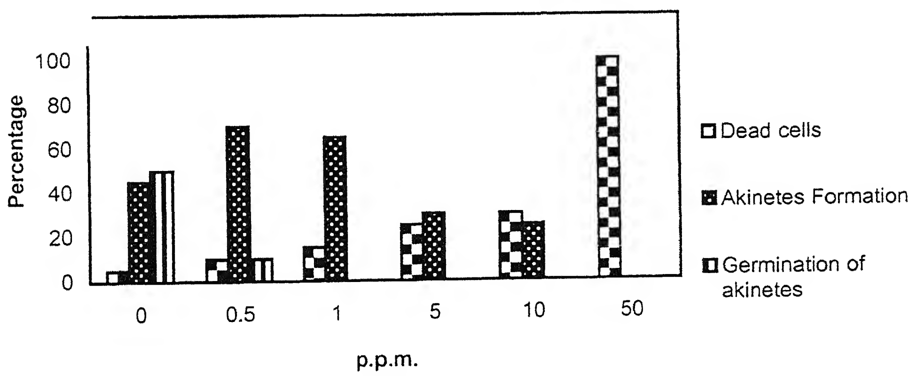


GRAPH- XII
EFFECTS OF MERCURIC CHLORIDE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

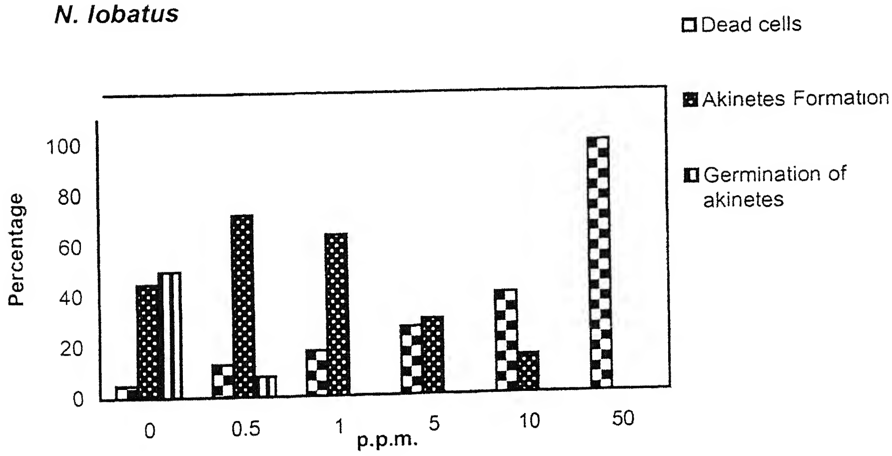
A. iyengarii



W. prolifica

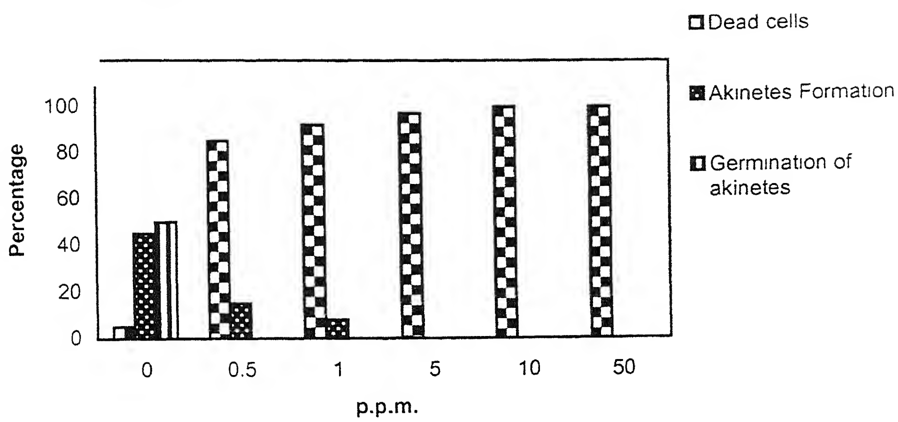


N. lobatus

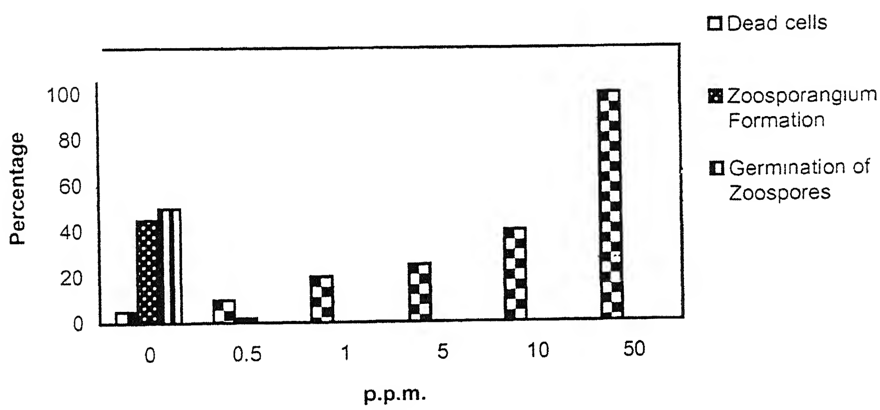


Contd.

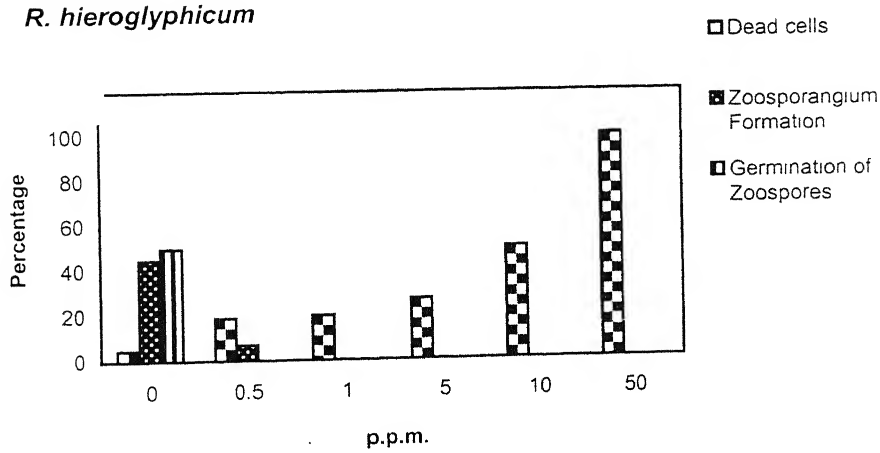
P. oedogonia



C. glomerata



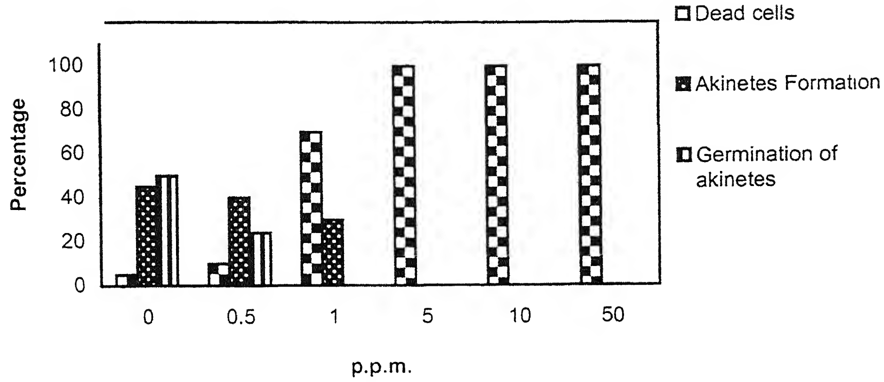
R. hieroglyphicum



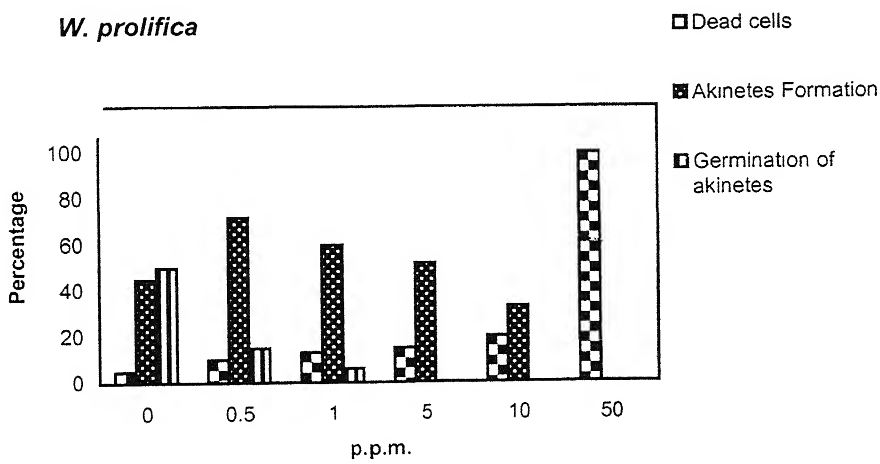
GRAPH- XIII

EFFECTS OF LEAD NITRATE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

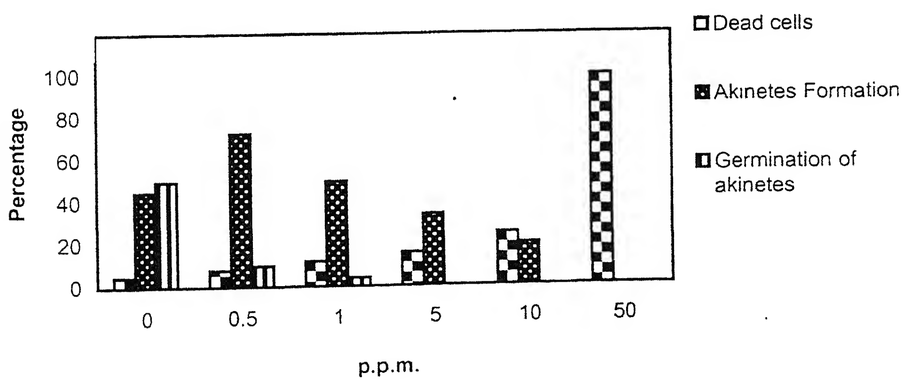
A. iyengarii



W. prolifica

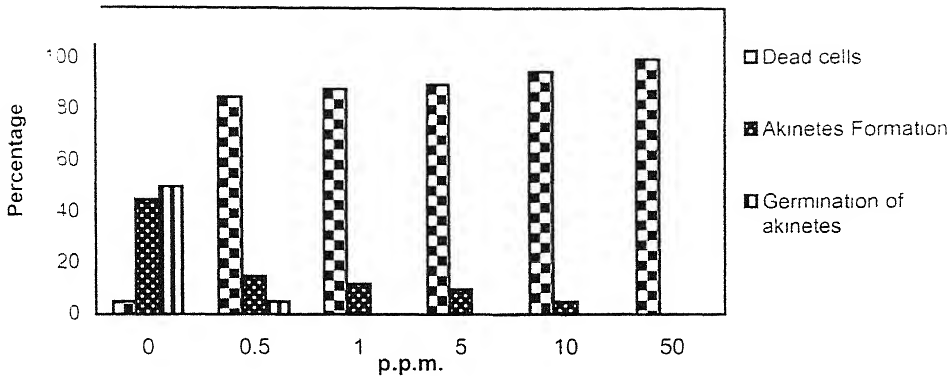


N. lobatus

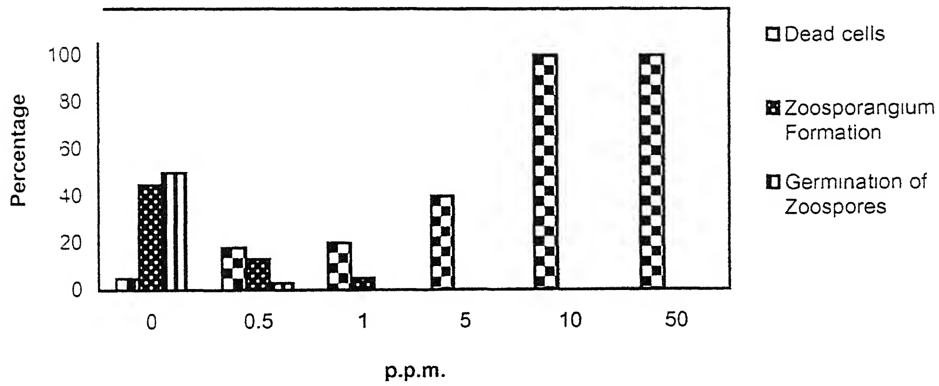


Contd.

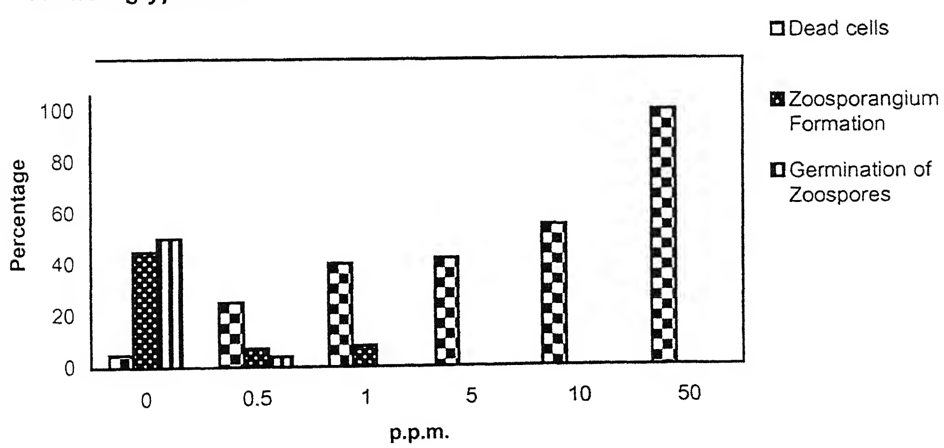
P. oedogonia



C. glomerata



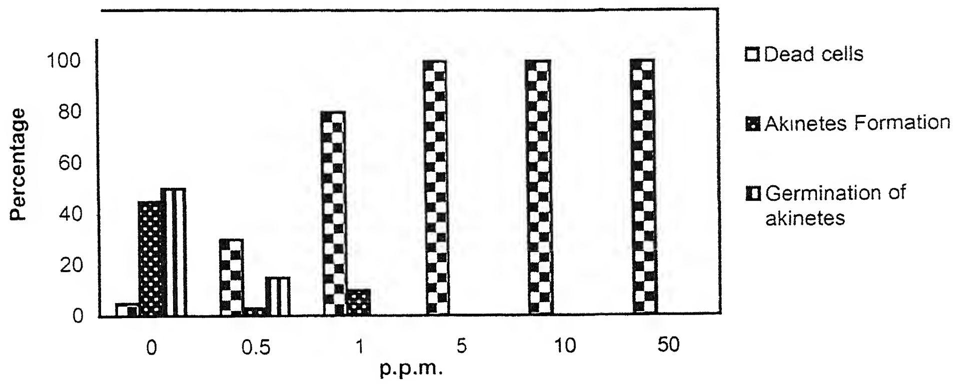
R. hieroglyphicum



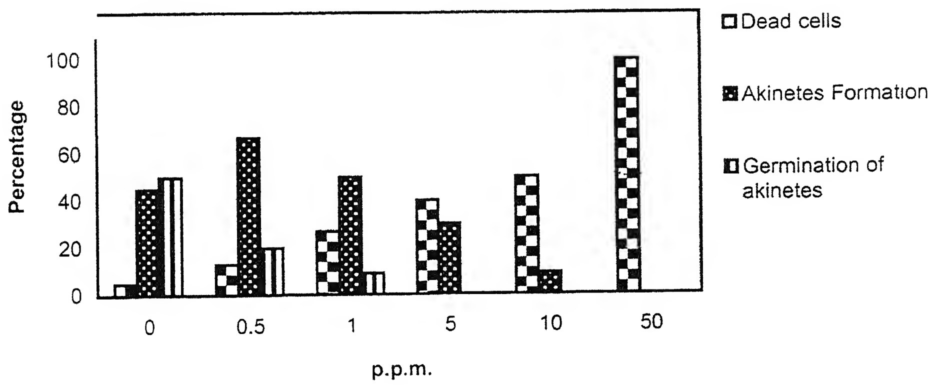
GRAPH- XIV

EFFECTS OF COBALT NITRATE ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

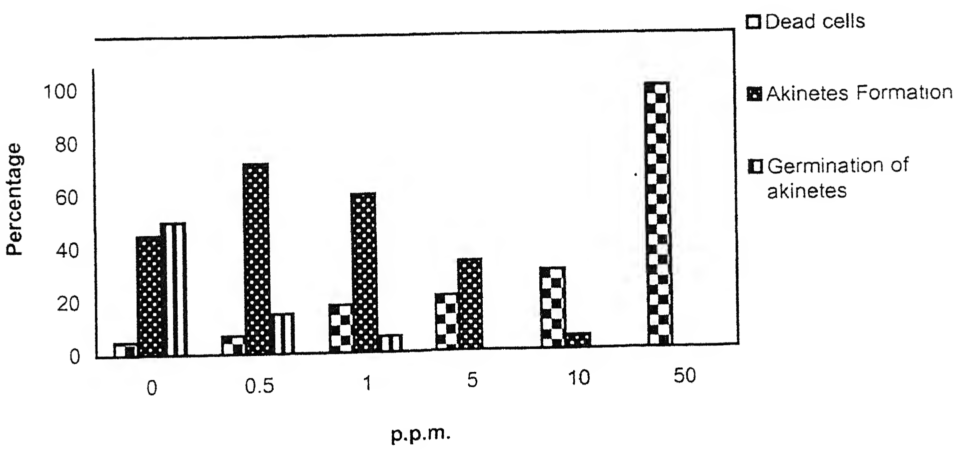
A. iyengarii



W. prolifica

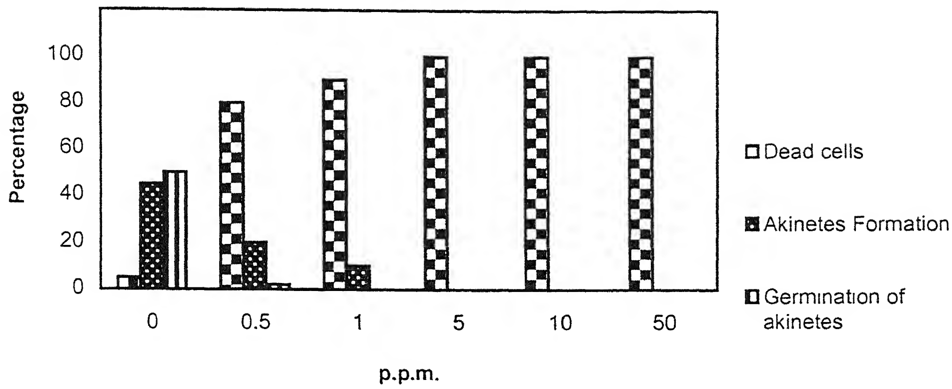


N. lobatus

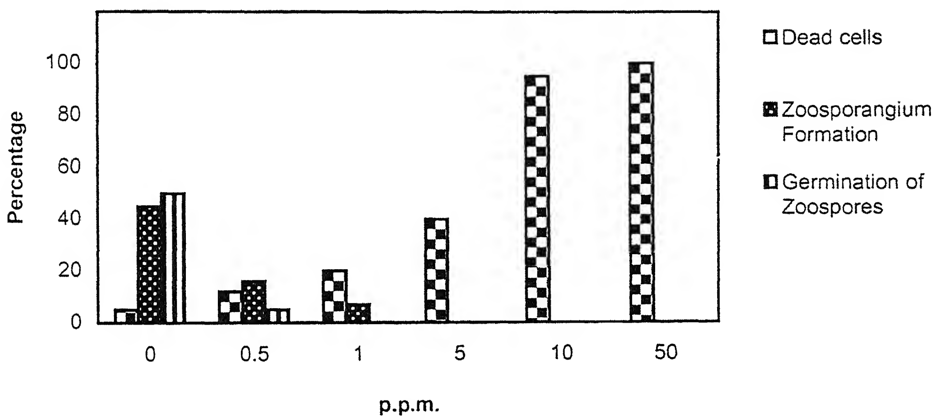


Contd.

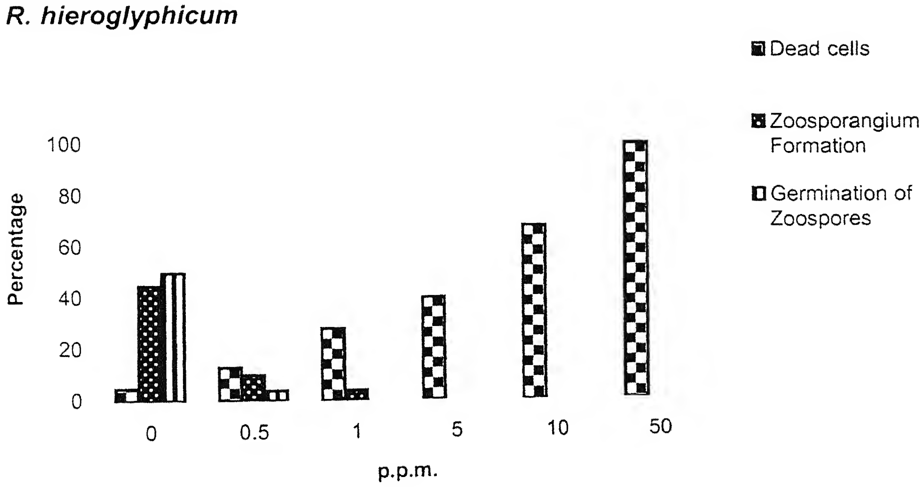
P. oegononia



C. glomerata



R. hieroglyphicum



CHAPTER VII

EFFECTS OF GROWTH HORMONES ON THE SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY AND GERMINATION OF AKINETES OR ZOOSPORES IN DIFFERENT ALGAE USED

CHAPTER VII

EFFECTS OF GROWTH HORMONES ON THE SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA, THEIR VIABILITY, AND GERMINATION OF AKINETES OR ZOOSPORES IN DIFFERENT ALGAE USED

Experimental work concerning the effects of Indole Acetic Acid (IAA) and Gibberellic Acid (GA) on the stimulation of algal growth is available (Saona, 1964; Jennings, 1968; Ahmad & Winter, 1968; Sundaralingam and Govindraj, 1977), but reports on the effects of plant growth hormones on the sporulation and spore germination in algae are scant.

Yin (1937) studied the effects of auxin on *Chlorella vulgaris* and observed an increase in cell size of alga following hormone treatment. Provasoli (1958) observed that IAA and kinetin at very low concentrations are effective to increase growth in *Ulva* thallūs. Kim and Greulach (1961) found that IAA and kinetin at certain levels favour the growth of *Oedogonium cardiacum* and *Chlorella pyrenoidosa*. Applications of gibberellic acid to cultures of *Scenedesmus* sp. and *Chlorella* sp. stimulated their cell division (Saona, 1964).

IAA at some low levels increased the formation of zoospores in *Ulothrix* (Conrad *et al.*, 1959), spores in *Derbesia* (Hustede, 1964) aplanospores in *Trebouxia* (Giles, 1970), akinetes in *Stigeoclonium* (Agrawal and Sarma, 1984), caps in *Acetabularia* (Driessche, 1984) and

Oogonia in *Oedogonium* (Singh and Chaudhary, 1988), but the same hormone (IAA) inhibited the production of sporangia in conchocelis phase of *Porphyra* (Dring, 1967). A delay in discharge of gametes was observed in *Fucus* following treatments with Napthalene acetic acid (Moss, 1967).

Gibberellic acid (GA_3) also at some low levels increased the formation of zoospores in *Ulothrix* (Conrad *et al.*, 1959), akinetes in *Stigeoclonium* (Agrawal and Sarma, 1984) and Oogonia in *Oedogonium* sp. (Singh & Chaudhary, 1988). Similarly, kinetin, at some low concentrations stimulated the formation of caps in *Acetabularia* (Spencer, 1968), and sexuality in *Chlamydomonas* in dark (Ishiura, 1976), but delayed the discharge of gametes in Fucus (Moss, 1967).

The present investigation deals with the effects of 3 Indole acetic acid (IAA) (Loba Chemical, Bombay); Gibberellic acid (GA_3) (Lubin Agrochemical, India); and Kinetin (6-furfurylaminopurine, Loba Chemic, India) on the survivability of vegetative cells in all algae used; formation and germination of akinetes in *Anabaena iyengarii, Westiellopsis prolifica, Nostochopsis lobatus* and *Pithophora oedogonia*; and on the formation of zoosporangia and germination of zoospores in *Cladophora glomerata* and *Rhizoclonium hieroglyphicum.*

METHODS

IAA, GA_3 and Kinetin were dissolved, separately, in a minimal volume of 80% ethanol and mixed slowly into a known amount of autoclaved and cooled culture medium so as to prepare the hormone

solution of desired concentrations. The range of concentrations of hormones used range between 0.1 to 500 p.p.m.. Controls were maintained containing the same amount of ethanol as were in those of hormones solutions used. The pH of the medium was adjusted to 7.5. Observations were made to determine the survivability of vegetative cells, formation of akinetes and zoosporangia, their viability and the germination of akinetes or zoospores.

(i) Survivability of vegetative cells and formation of akinetes and zoosporangia

In order to observe the effects of different growth hormones on the survivability of vegetative cells and the formation of akinetes and zoosporangia in different algae used, the seven-day-old actively growing vegetative filaments of all algae were used as a source of inoculum. Controls were maintained in standard BG 11. All inoculated culture tube were placed in the culture chamber and were examined on 60 days of inoculation in order to determine the percentage of dead cells, if any, in all algae used; that of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*; and that of zoospores in *C. glomerata* and *R. hieroglyphicum* with respect to total number of about 5000-6000 vegetative cells scored.

(ii) Viability of akinetes or zoosporangia formed

In order to determine the viability of akinetes or zoosporangia formed in presence of hormone, they were harvested from the hormone solution, respectively after 60 and 45 days of inoculation and were inoculated into basal medium and placed in culture chamber. Akinetes or

zoosporangia harvested from the basal medium containing no hormone served as controls. The percentage germination of akinetes or that of empty zoosporangia which have released zoospores was estimated on 15 day of inoculation.

(iii) Germination of akinetes or zoospores

Mature akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and freshly released zoospores of *C. glomerata* (adhering to coverslips) and *R. hieroglyphicum* (adhering to parent filaments), formed in standard basal medium, were separately inoculated into culture tubes containing different concentrations of hormones used. Controls were maintained in standard basal medium. All inoculated culture tubes were placed in culture chamber under controlled culture condition. The percentage germination of akinetes or that of zoospores was determined by counting about 3000 akinetes or zoospores on 15 days after inoculation.

RESULT AND DISCUSSION

(i) Survivability of vegetative cells, formation of akinetes or zoosporangia and their viability

Maximum survivability of *A. iyengarii* vegetative cells was observed at 0.1 p.p.m. of any of three hormones used. The survivability of alga decreased slightly at 1 p.p.m. of any of hormones used, but was still more than that in control. (Table V, Graph XV to XVII). However, it decreased as the concentration of IAA and GA_3 increased from 10 to 100 p.p.m., and Kinetin from 1 to 10 p.p.m.. At 500 p.p.m. of IAA and GA_3 and at 100

p.p.m. of Kinetin, all vegetative cells of the algae died. (Table V, Graph XV to XVII). Akinete formation in *A. iyengarii* was cent per cent at 0.1 p.p.m. of any of hormones used. It was slightly more than control or close to control (where it was about 85%) at 1 p.p.m. of any of hormones used. However, it decreased or suppressed altogether as the concentration of hormones increased from 1 to 100 or 500 p.p.m.. (Table V, Graph XV to XVII).

Akinetes formed at 0.1 and 1.0 p.p.m. of all hormones used were viable, similar to controls, but those formed at 10 p.p.m. IAA and 100 p.p.m. GA_3 were viable only by about 5-10% as compared to controls. Akinetes were not viable at all when formed at 100 p.p.m. IAA or 10 p.p.m. Kinetin. Exogeneously applied IAA and GA produced an increasing growth response in various algae only upto some concentrations and thereafter an inhibition in growth was observed (Conrad *et al.*, 1959; Saona, 1964; Sunderalingam & Govindraj, 1977). IAA and GA_3 upto 0.1 and 10 p.p.m., respectively, did not bring any change in survival of vegetative colony in *Stigeoclonium pascheri*, but beyond these levels proved inhibitory to colony survival. (Agrawal and Sarma, 1984).

The survivability of vegetative cells in *W. prolifica* and *N. lobatus* was maximum at 0.1 p.p.m. of IAA and GA_3 and at both 0.1 and 1 p.p.m. of Kinetin as compared to controls, but it decreased or inhibited altogether as the concentration of any of these hormones increased. All vegetative cells of both algae died at 500 p.p.m. of IAA, GA_3 and Kinetin. In both algae, akinete formation was cent per cent or close to it at 0.1 p.p.m. of IAA and GA_3 and at both 0.1 and 1.0 p.p.m. of Kinetin (Table V, Graph XV

to XVII), however it got reduce or suppress altogether at concentrations beyond 10 p.p.m. of any of hormones used. (Table V, Graph XV to XVII). Sarma and Tripathi (1974) showed that in *Oedogonium acmandrium*, growth of alga was accelerated upto 10 p.p.m. GA_3 but was inhibited as the concentrations increased from 12.5 to 100 p.p.m..

Akinetes formed upto 1.0 p.p.m. IAA, 10 p.p.m. GA_3 and 1.0 p.p.m. Kinetin were viable as those of controls but those formed at 10 p.p.m. IAA, 100 p.p.m. GA_3 and 10 p.p.m. Kinetin were viable by about 5-10% only. Akinetes formed at 100 p.p.m. IAA and Kinetin were not viable at all (Table V).

The survivability of *P. oedogonia* vegetative cells decreased as the concentrations of IAA increased from 0.1 to 10 p.p.m., GA_3 from 0.1 to 1.0 p.p.m., and Kinetin from 0.1 to 10 p.p.m.. All vegetative cells of *P. oedogonia* died at 100 p.p.m. of IAA, 10 p.p.m. of GA_3 and 100 p.p.m. of Kinetin (Table V, Graph XV to XVII). The formation of akinetes in *P. oedogonia* was inhibited at 0.1 p.p.m. of IAA, 0.1 and 1.0 p.p.m. of GA_3, and at 1.0 and 10 p.p.m. of Kinetin. The akinete formation in *P. oedogonia* was almost equal to control at 0.1 p.p.m. of Kinetin but the process did not occur at all beyond 0.1 p.p.m. of IAA, 1 p.p.m. of GA_3 and 10 p.p.m. of Kinetin (Table V, Graph XV to XVII).

Akinetes of *P. oedogonia* formed at 0.1 p.p.m. kinetin were viable similar to those of control, but those formed at 0.1 p.p.m. IAA, 0.1 and 1 p.p.m. GA_3 and at 1.0 p.p.m. Kinetin were only 5-10% viable. Akinetes of

P. oedogonia formed at 10 p.p.m. of kinetin were not viable at all (Table V).

The survivability of *C. glomerata* and *R. hieroglyphicum* vegetative cells formed at 0.1 p.p.m. of any of three growth hormones used was almost equal to that of controls, but decreased first slowly and thereafter rapidly as the concentrations of IAA increased from 1 to 10 p.p.m., and GA_3 and Kinetin from 1.0 to 100 p.p.m.. Both algae died at 100 p.p.m. of IAA and at 500 p.p.m. of GA_3 and Kinetin. The zoosporangia formation in *C. glomerata* and *R. hieroglyphicum* occurred only upto 1 p.p.m. of IAA, 100 p.p.m. of GA_3 and 10 p.p.m. of Kinetin. (Table V, Graph XV to XVII). In both algae, zoosporangia formation increased at both 0.1 and 1.0 p.p.m. of IAA, GA_3 and Kinetin as compared to controls but it decreased as the concentrations of these hormones increased further and did not occur at all beyond 1 p.p.m. of IAA and 100 p.p.m. of GA_3 and 10 p.p.m. of Kinetin. (Table V, Graph XV to XVII).

In both algae, viability of zoosporangia was severely decreased when formed at 10 and 100 p.p.m. GA_3 and at 10 p.p.m. of kinetin but was not affected at all when formed at 0.1 and 1.0 p.p.m. of any of three hormones used (Table V).

(ii) Akinete and zoospore germination

IAA, at 0.1 and 1.0 p.p.m. of concentrations increased at various levels the akinete and zoospore germination in present algae, but when present at 10 and 100 p.p.m. levels, retarded the germination of akinetes

as well as of zoospores. (Table V, Graph XV). IAA at certain levels also stimulated the germination of zygospores in *Hydrodictyon reticulatum* (Rowan, 1937). In *Vaucheria* sp., zoospores germination was promoted by IAA and typtophan (Hustede, 1957). In *Ulva* sp. IAA in presence of kinetin induced large number of germlings from dormant green cells (Provasoli, 1958). Agrawal and Sarma (1984) observed that IAA at 0.01 and 0.1 p.p.m. concentrations increased the percentage akinete germination in *Stigeochonium pascheri.*

GA_3 from 0.1 to 100 p.p.m. was favourable to akinete germination in *A. iyengarii* and from 0.1 to 500 p.p.m. to akinete germination in *W. prolifica*, *N. lobatus* and *P. oedogonia* and to zoospore germination in *C. glomerata* and *R. hieroglyphicum.* (Table V, Graph XVI). In all these cases, the germination of akinetes or zoospores in present algae was either equal to or more than controls. (Table V, Graph XVI). Agrawal and Sarma (1984) observed that GA_3 at 0.01 to 500 p.p.m. increased the percentage akinete germination in *Stigeoclonium pascheri.*

Akinetes or zoospores germination in all algae studied was enhanced at 0.1 p.p.m. of kinetin. (Table V, Graph XVII). Kinetin at 1 p.p.m. did not produce much change to akinete germination in *A. iyengarii*, *W. prolifica* and *N. lobatus* and to zoospore germination in *C. glomerata* and *R. hieroglyphicum* but severely decreased akinete germination in *A. iyengarii*, *W. prolifica* and *N. lobatus* and inhibited altogether the akinete germination in *P. oedogonia*. At 10 p.p.m., it decreased akinete germination in *P. oedogonia* and zoospore germination in *C. glomerata*

and *R. hieroglyphicum*. (Table V, Graph XVII). No akinete or zoospore germination was observed in any algae studied at 100 p.p.m. of kinetin. (Table V, Graph XVII).

Thus, at low levels of either 0.1 or 1 p.p.m. all three growth hormones enhanced survivability of vegetative cells, formation of akinetes and zoosporangia and germination of akinetes or zoospores in present algae, but beyond that they proved inhibitory. However GA_3 could promote germination of akinetes or zoospores in all algae studied even upto 100 or 500 p.p.m. levels.

Table V : Percentage formation of dead cells (D) in all algae; formation of akinetes (A) and germination of akinetes (AG) in *A. iyengarii*, *W. prolifica*, *N. lobatus* and *P. oedogonia*; and on zoosporangia formation (Z) and germination of zoospores (ZG) in *C. glomerata* and *R. hieroglyphicum* at different concentration of plant growth hormones used. Formation of dead cells, akinetes and zoosporangia were counted on 60 days of inoculation of vegetative filaments while akinete or zoospore germination on 15 days after inoculation of akinetes or zoospores[a]

Plant growth hormones (in ppm)		Algae																	
		A. iyengarii			*W. prolifica*			*N. lobatus*			*P. oedogonia*			*C. glomerata*			*R. hieroglyphicum*		
		D	A	AG	D	A	AG	D	A	AG	D	A	AG	D	Z	ZG	D	Z	ZG
Control		15	85	50	4	70	52	2	80	50	10	36	67	4	40	48	7	44	52
IAA	0.1	0	100[b]	70	0	100[b]	80	0	90[b]	74	40	20[c]	80	6	70[b]	60	5	80[b]	72
	1.0	5	85[b]	50	2	92[b]	60	10	75[b]	60	55	0	85	10	60[b]	75	20	65[b]	60
	10	40	20[c]	5	10	15[c]	10	35	10[c]	4	60	0	10	20	0	0	40	0	0
	100	80	5[d]	0	30	10[d]	0	45	4[d]	0	100	0	-	100	0	0	100	0	0
	500	100	0	0	100	0	0	100	0	0	-	-	-	-	-	-	-	-	-
GA_3	0.1	0	100[b]	74	0	100[b]	68	0	90[b]	70	15	25[c]	80	5	50[b]	59	0	70[b]	65
	1.0	2	80[b]	70	2	80[b]	74	7	80[b]	82	20	3[c]	75	20	45[b]	64	10	60[b]	70
	10	30	40[b]	60	15	60[b]	80	10	50[b]	89	100	0	89	20	30[c]	78	20	45[c]	85
	100	20	15[c]	55	30	45[c]	70	50	32[c]	72	100	0	70	40	10[c]	60	35	20[c]	55
	500	100	0	0	100	0	60	100	0	65	100	0	70	100	0	47	100	0	50

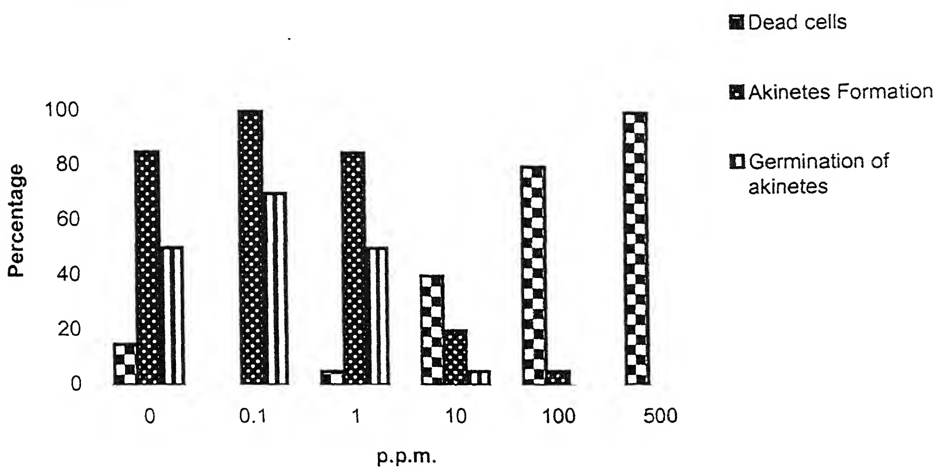
Kinetin	0.1	0	100[b]	75	0	100[b]	70	0	100[b]	65	20	40[b]	70	7	55[b]	60	6	60[b]	65
	1.0	2	95[b]	60	0	100[b]	55	0	100[b]	64	22	25[c]	6	15	45[b]	50	12	50[b]	50
	10	50	10[d]	10	5	30[c]	20	7	24[c]	15	30	5[d]	0	20	7[c]	0	11	12[c]	0
	100	100	0	0	10	15[d]	0	15	12[d]	0	100	0	0	30	0	0	25	0	0
	500	-	-	-	100	0	0	100	0	0	-	-	-	100	0	0	100	0	0

[a] Data represent mean of three replicates.
[b] Viability similar to those harvested from basal medium.
[c] Viability reduced to 5-10% as compared to those harvested from basal medium.
[d] No viability at all.

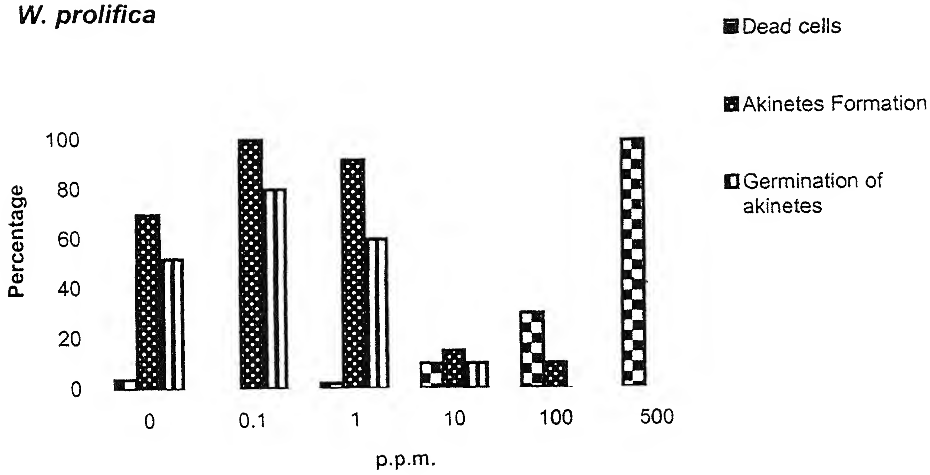
GRAPH- XV

EFFECTS OF IAA ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

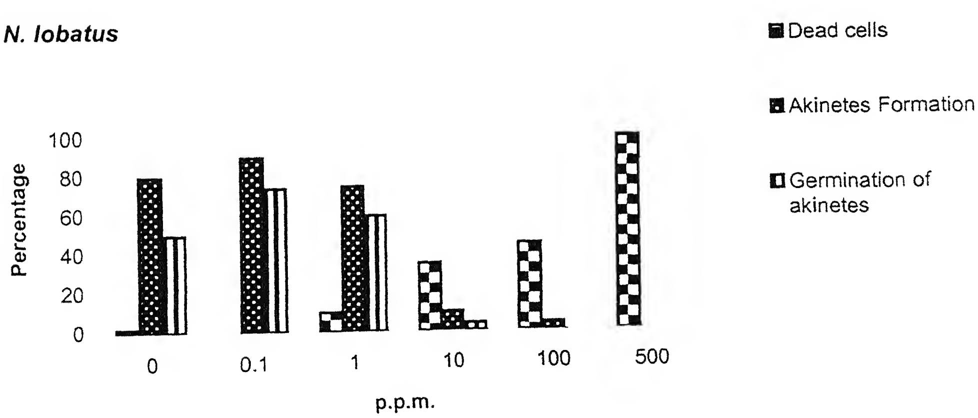
A. iyengarii



W. prolifica

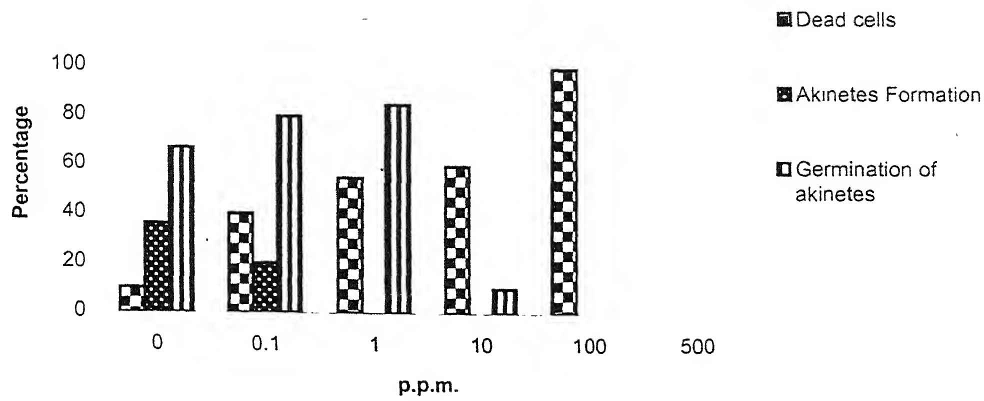


N. lobatus

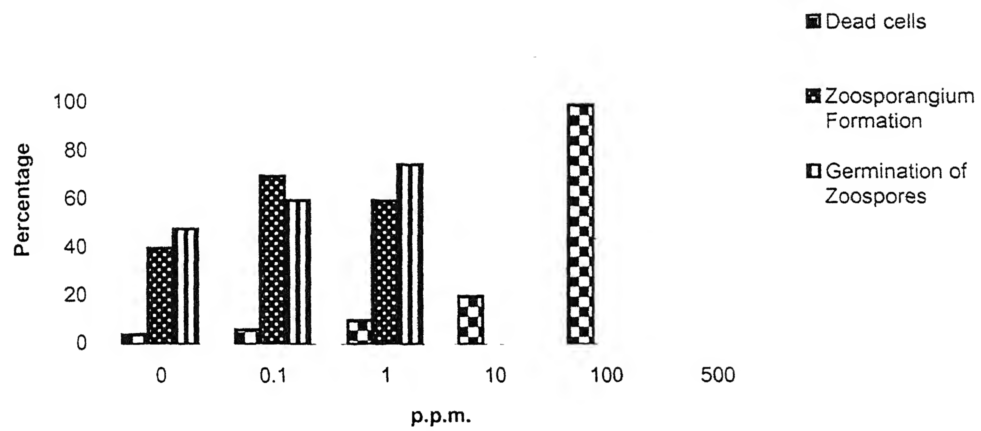


Contd.

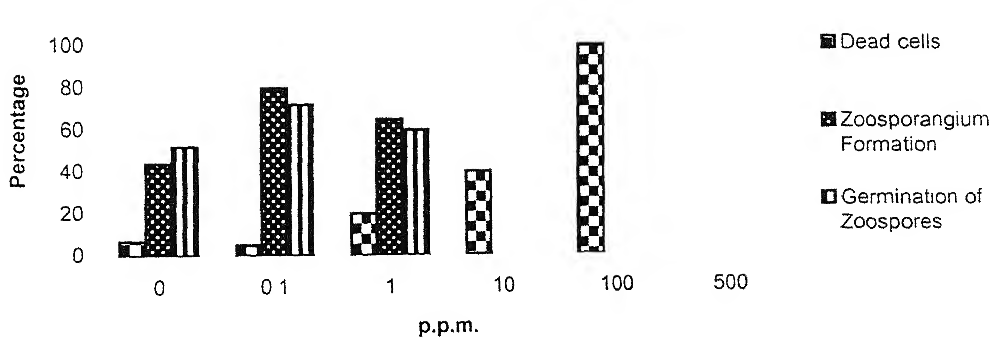
P. oedogonia



C. glomerata



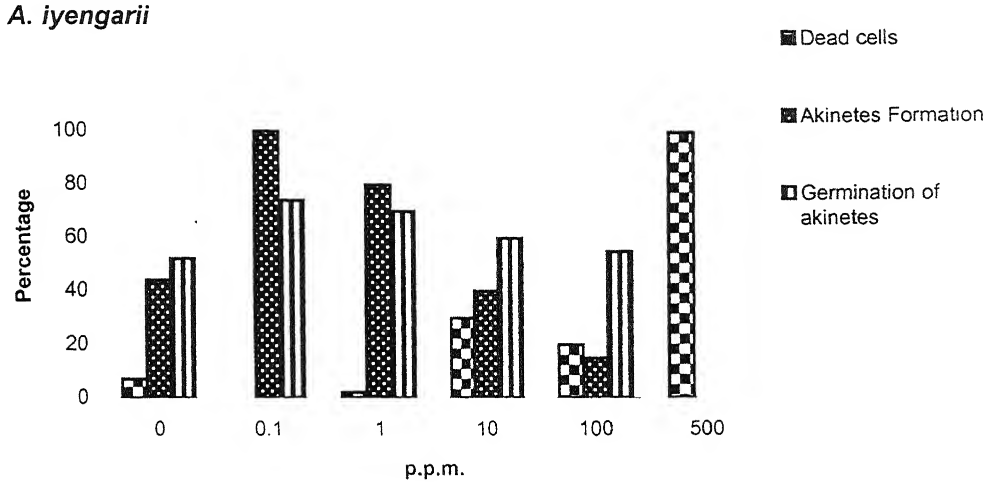
R. hieroglyphicum



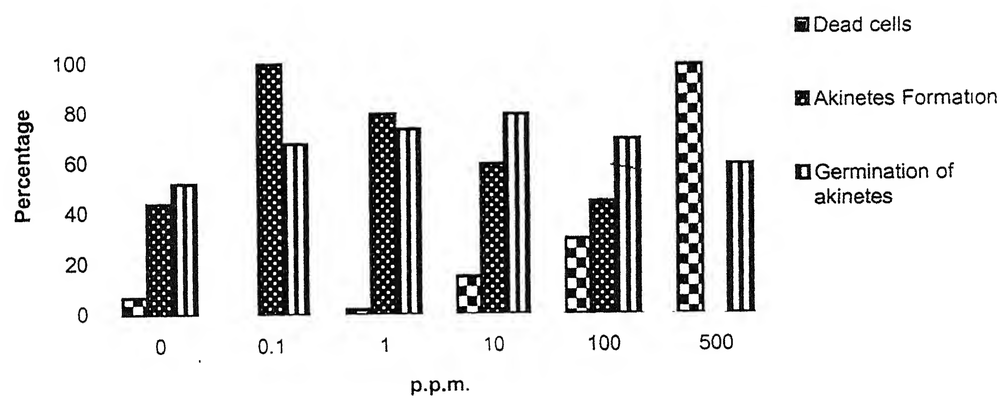
GRAPH- XVI

EFFECTS OF GA_3 ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

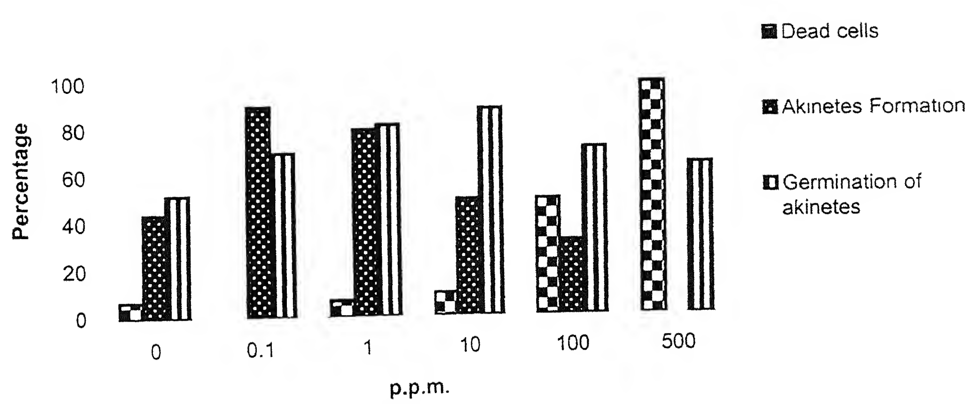
A. iyengarii



W. prolifica

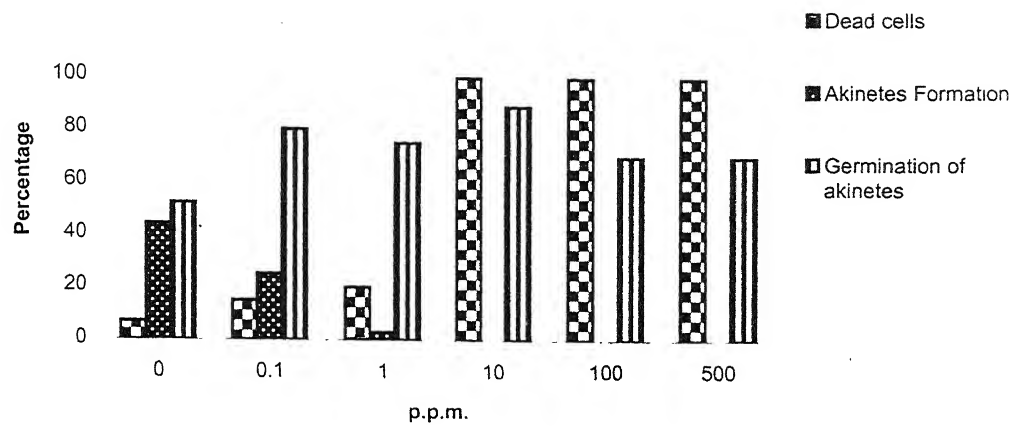


N. lobatus

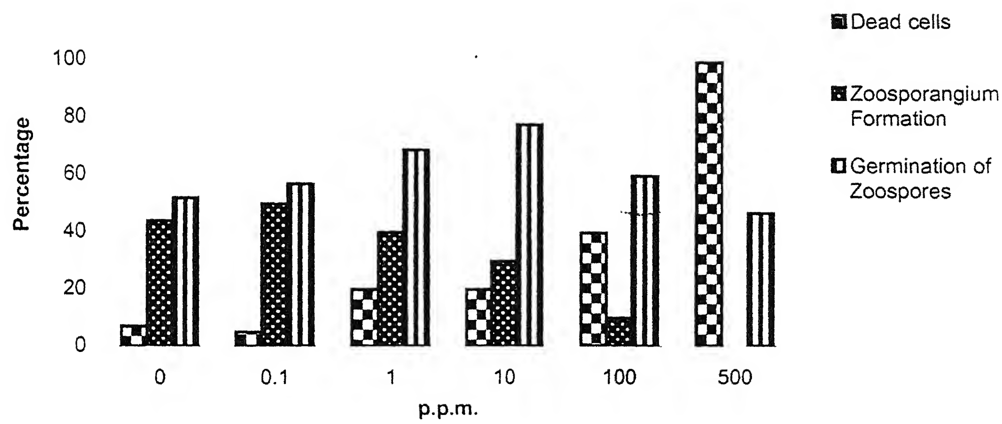


Contd.

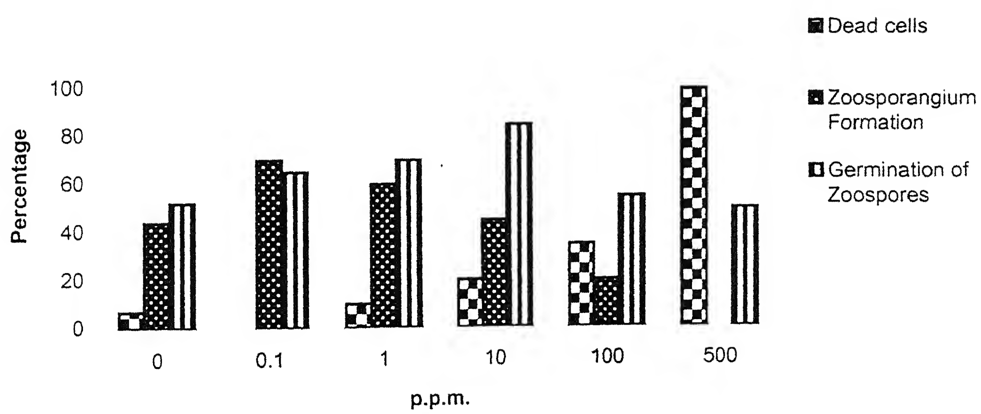
P. oedogonia



C. glomerata



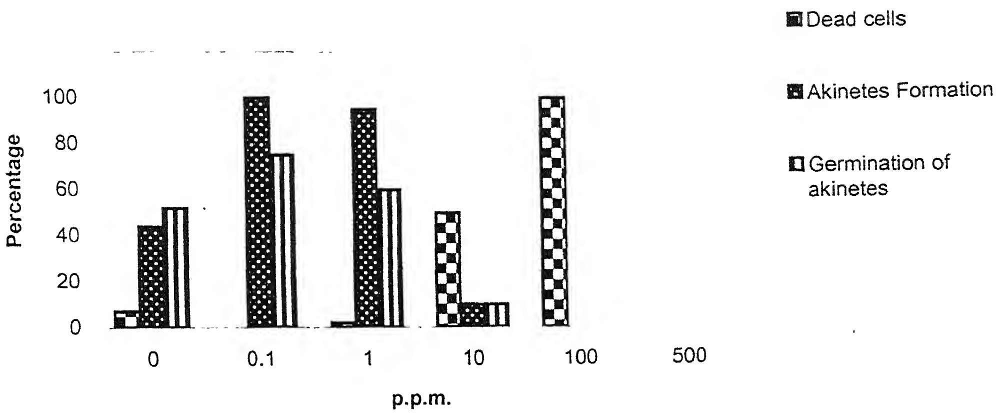
R. hieroglyphicum



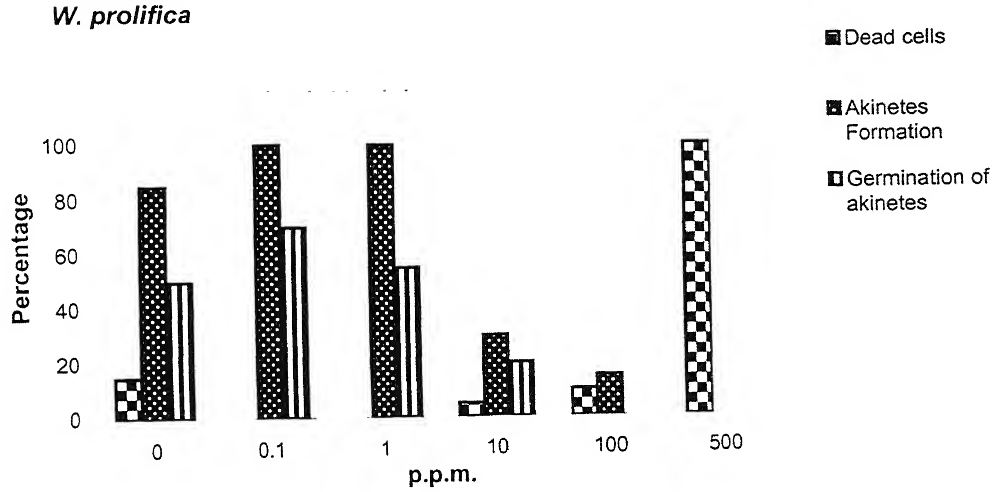
GRAPH- XVII

EFFECTS OF KINETIN ON SURVIVABILITY OF VEGETATIVE CELLS, FORMATION OF AKINETES OR ZOOSPORANGIA AND GERMINATION OF AKINETES OR ZOOSPORES IN ALGAE STUDIED

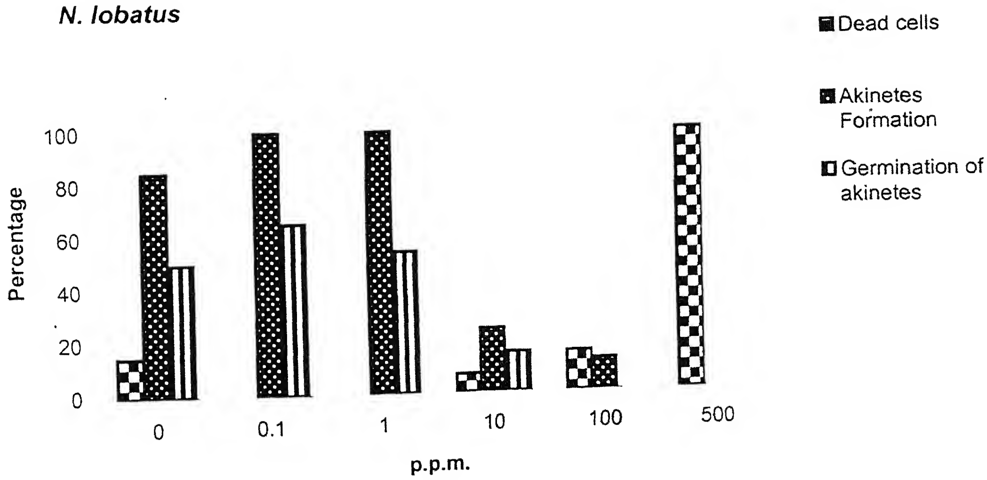
A. iyengarii



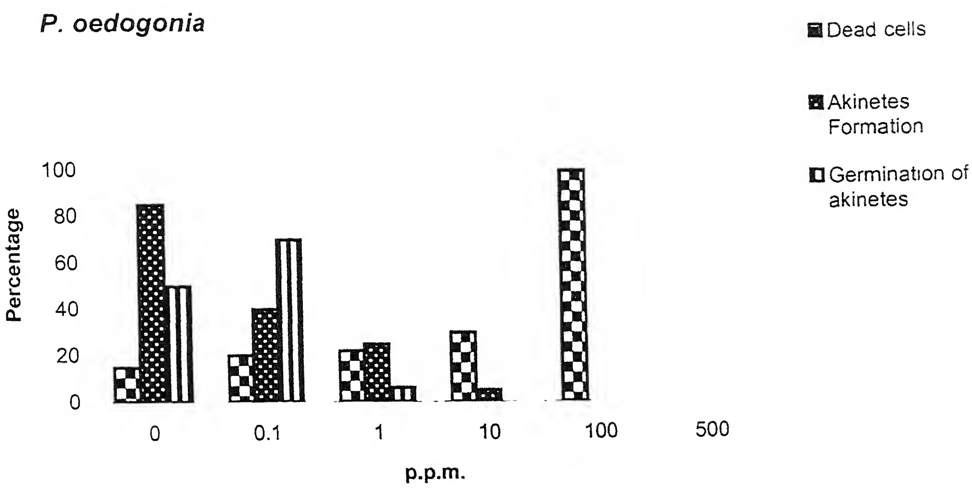
W. prolifica

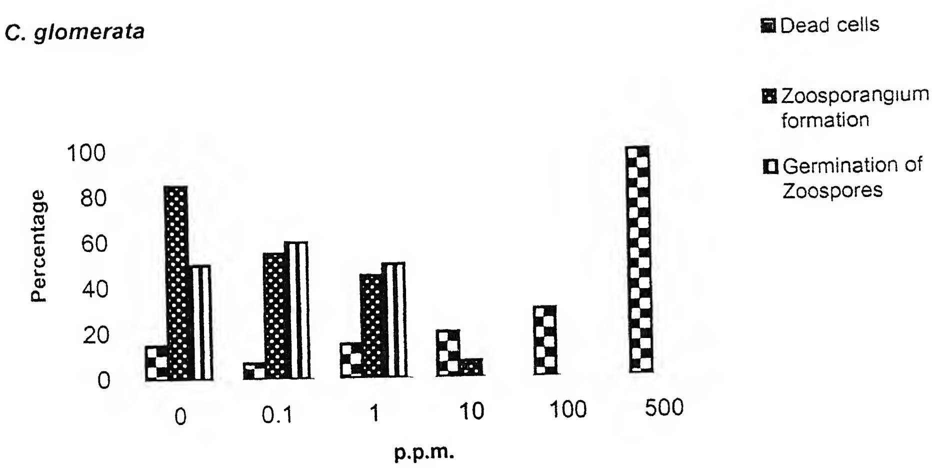


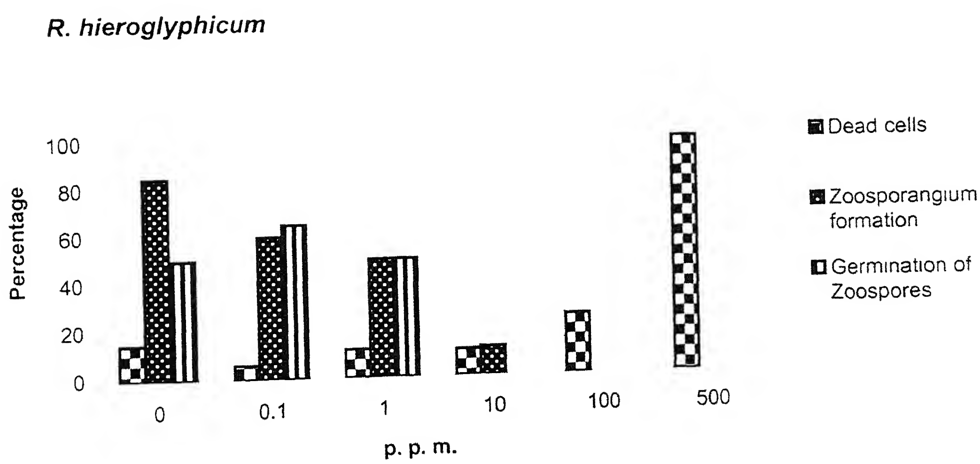
N. lobatus



Contd.


P. oedogonia
Dead cells
Akinetes Formation
Germination of akinetes
100
80
60
40
20
0
Percentage
0
0.1
1
10
100
500
p.p.m.


C. glomerata
Dead cells
Zoosporangium formation
Germination of Zoospores
100
80
60
40
20
0
Percentage
0
0.1
1
10
100
500
p.p.m.


R. hieroglyphicum
Dead cells
Zoosporangium formation
Germination of Zoospores
100
80
60
40
20
0
Percentage
0
0.1
1
10
100
500
p. p. m.

CHAPTER VIII

SUMMARY AND CONCLUSION

CHAPTER VIII

SUMMARY AND CONCLUSION

The present study was aimed to find out that to what extent chemical stresses like amendments in nutrients concentrations or pH value of the basal medium, or the presence of different pesticides like carbofuran, 2,4-D, dithane, phorate or bavistin or different heavy metals like copper, zinc, mercury, lead or cobalt, or growth hormones like IAA, GA_3 and Kinetin in the basal medium produce changes to the survivability of vegetative cells, formation of akinetes in *Anabaena iyengarii* var. tenuis RAO, *Westiellopsis prolifica* JANET, *Nostochopsis lobatus* WOOD, *Pithophora oedogonia* (MONT) WITTROCK; zoosporangia in *Cladophora glomerata* (L) KUETZING and *Rhizoclonium hieroglyphicum* (C.A.Ag). KUETZING, their viability and germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or zoospores in *C. glomerata* and *R. hieroglyphicum.*

These algae were selected for the present study because most of them were collected from local habitats and all of them grew and reproduce well in culture. *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* formed akinetes abundantly in culture, while *C. glomerata* and *R. hieroglyphicum*, the zoosporangia. Akinetes or zoospores of all algae could germinate prolifically in culture.

All algae were grown in BG 11 medium (Stanier *et al.*, 1971). Their cultures were maintained in controlled culture condition at 22±1°C temperature and 2 klux light intensity for 16 hours a day. In order to

maintain them in actively growing condition, subculturing was done after every 10-15 days of inoculation.

Effects of nutrients present in basal medium on the survivability of vegetative cells, differentiation of akinetes or zoosporangia, their viability, and germination of akinetes or zoospores

Methodology

Seven-day-old actively growing young vegetative filaments of all algae were inoculated into culture tubes containing the amended BG 11 medium (the medium was amended either by omitting or increasing to 5 or 10 fold level of either a single nutrient compound in question or all of them together). The controls were maintained in standard basal medium. The survivability of vegetative cells in all algae, and the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and that of zoosporangia in *C. glomerata* and *R. hieroglyphicum* were determined by counting the number of dead cells, if any, and that of akinetes or zoosporangia with respect to total number of about 5000-6000 vegetative cells at time interval of 30 and 60 days of inoculation.

The viability of akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or that of zoosporangia of *C. glomerata* and *R. hieroglyphicum* formed under such amended chemical conditions was also determined with respect to those formed in basal medium, and for that the mature akinetes or zoosporangia bearing filaments formed under such conditions were harvested on 60 and 45 days after inoculation,

respectively, washed with distilled water and inoculated into basal medium and placed in culture chamber.

In order to see the effects of altered chemical composition on akinetes and zoospores germination, the akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and zoospores of *C. glomerata* (adhering to coverslips) and *R. hieroglyphicum* (adhering to parent filaments) formed in basal medium were inoculated in amended medium and the percentage germination of akinetes or zoospores was determined by counting 2500-3000 akinetes or zoospores on 15 days of inoculation.

Findings and conclusion

1. The survivability of vegetative cells in all algae, the differentiation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or that of zoosporangia in *C. glomerata* and *R. hieroglyphicum*, their viability, and the germination of akinetes or zoospores, all were best in standard basal medium and were adversely affected at various levels by nutrients amendments of basal medium studied.
2. Omission of any of single nutrient compound from the basal medium was less effective to change survivability, reproduction and spore germination in any of algae studied than was that of all nutrients from the basal medium (i.e. double distilled water). But all algae could survive and reproduce to some extent even in double distilled water, indicating that they require very limited amounts of

extrabiotic nutrients for optimal performance of various life cycle stages.

Nitrogen limitation was somewhat less effective to produce changes in survivability and reproduction of blue-green algae than in green algae and this might be due to nitrogen fixation ability of the blue-green algae.

3. Media containing even 5-fold level of an individual nutrient compound proved inhibitory to all life cycle stages of different algae studied, but the toxicity level was much increased when the concentration of all nutrients was increased to 5-fold levels. Thus increasing nutrients concentrations beyond that present in basal medium did not help to improve survival or reproduction in algae, but proved inhibitory to all life cycle stages of algae studied.

4. *A. iyengarii* was the most sensitive alga, while *R. hieroglyphicum*, the most resistant to any of nutrients amendments studied, and this might be due to more delicate nature of *A. iyengarii* filaments than rest of other algae studied.

5. The formation of zoosporangia, their viability and germination of zoospores in *C. glomerata* and *R. hieroglyphicum*, all were more sensitive to nutrients amendments of any levels than were the formation, viability and germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*.

Effects of pH on the survivability of vegetative cells, formation of reproductive structures, their viability, and germination of reproductive cells in algae studied.

Methodology

Basal medium was adjusted to different pH levels of 3.5 to 11.0 as determined through measurement with Toshniwal digital pH meter, prior to autoclaving either by adding 1N HCl or 2% NaOH solutions. In order to observe the effects of different pH levels of the medium on the survivability of vegetative cells and formation of akinetes or zoosporangia or germination of akinetes or zoospores, the seven-day-old actively growing filaments of all algae or mature akinetes of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or freshly released zoospores of *C. glomerata* and *R. hieroglyphicum* formed in basal media at pH 7 were separately inoculated into culture tubes containing media of different pH levels ranging between 3.5 to 11. Survivability of vegetative cells, and formation of reproductive structures and germination of akinetes or zoospores were determined as usual. The viability of akinetes or zoosporangia formed at extremes of pH was also determined.

Findings and Conclusion

1. Survivability of vegetative cells in all algae, the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and the zoosporangia in *C. glomerata* and *R. hieroglyphicum*, their viability, and the germination of akinetes and zoospores, all were observed to be optimum both at pH 7 as well as 8.

2. The survivability of vegetative cells in all algae and the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia,* their viability, and the formation of zoosporangia in *C. glomerata* and *R. hieroglyphicum*, their viability, and the germination of akinetes and zoospores in algae studied, all decreased at various levels as the pH of the medium was lowered from 7.0 to 4.0 or increased from 8.0 to 11.0.
3. All vegetative cells of *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* died at pH 3.5 without forming any akinete, but those of *C. glomerata* and *R. hieroglyphicum* could survive the same pH level to some extent but did not differentiate into any zoosporangium. Thus all blue-green algae studied as well as green alga *P. oedogonia* were slightly more sensitive to acidic pH than were green algae *C. glomerata* and *R. hieroglyphicum.*
4. Among blue-green algae studied, *A. iyengarii* was the most sensitive alga to extremes of pH; while *W. prolifica* and *N. lobatus,* more or less equally most resistant, and this might be due to differences in their body structures. Among green algae, *P. oedogonia* was the most sensitive alga to extremes of pH, while *R. hieroglyphicum*, the most resistant, and this might be probably due to differences in their cell wall thickness or size of cells. *P. oedogonia* possess thin wall, large size cells than *R. hieroglyphicum*.
5. Both the processes of zoosporangia formation and zoospore germination in *C. glomerata* and *R. hieroglyphicum* were

comparatively more sensitive to pH extremes than were those of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*. This indicates that akinetes were more resistant to pH extremes than zoosporangia or zoospores.

Effects of Pesticides on the survivability of vegetative cells, differentiation of akinetes or zoosporangia, their viability and germination of akinetes or zoospores

Methodology

The graded amounts of different pesticides viz., carbofuran, 2,4-D, dithane, phorate or bavistin were mixed, separately, with basal medium, pH was adjusted to 7.5, prior to autoclaving so as to prepare the solutions of desired concentrations of 1 to 50 ppm. The seven-day-old actively growing vegetative filaments of all algae obtained from basal medium, washed carefully with the media of particular pesticide level, were inoculated, separately, into culture tubes containing that respective media, and placed in culture chamber. Controls were maintained in the standard BG 11 medium. Survivability of vegetative cells in all algae, the formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or zoosporangia in *C. glomerata* and *R. hieroglyphicum*, their viability, and the germination of akinetes and zoospores were determined as usual.

Findings and Conclusion

1. Survivability of vegetative cells in all algae, formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or zoosporangia in *C. glomerata* and *R. hieroglyphicum*, their viability

and germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* or zoospores in *C. glomerata* and *R. hieroglyphicum*, all were maximum in control medium containing no pesticide, but the presence of any of pesticides in the basal medium at 1-50 ppm concentrations decreased progressively at various levels or inihibited altogether all of the above processes in all algae studied. Thus all life cycle stages of present algae studied behaved similarly to any of pesticides used.

2. Among blue-green algae, *W. prolifica* and *N. lobatus* were more or less equally most tolerant algae since they could survive and differentiate into akinete even at 50 p.p.m. of any of all pesticides used, while among green algae, *R. hieroglyphicum*, the most. *A. iyengarii* and *P. oedogonia* were most sensitive algae to most of pesticides used than were rest of other algae studied.
3. Both the processes of zoosporangia dehiscence as well as zoospore germination in *C. glomerata* and *R. hieroglyphicum* were very sensitive to all pesticides used. Since none of their zoosporangia dehisce or zoospores germinate even at 1 ppm of any of pesticides used.

Effects of heavy metals on the survivability of vegetative cells, formation of akinetes or zoosporangia, their viability, and germination of akinetes or zoospore in algae studied

Methodology

The graded amounts of different heavy metals viz., copper sulphate, zinc oxide, mercuric chloride, lead nitrate or cobalt nitrate were

mixed, separately with basal medium, and pH adjusted to 7.5 prior to autoclaving, in order to prepare heavy metal solutions of desired concentrations of 0.5 to 50 ppm. Seven-day-old actively growing vegetative filaments of different algae were separately inoculated into different concentrations of all heavy metals solutions. Controls were maintained in basal medium. The survivability of vegetative cells, formation of akinetes and zoosporangia, their viability, and the germination of akinetes or zoospores, all were determined as usual.

Findings and Conclusion

1. The survivability of vegetative cells, formation of akinetes and zoosporangia, their viability and germination of akinetes or zoospores in all algae studied were maximum in control basal medium containing no heavy metal and were found to decrease at various levels or suppressed totally as concentrations of different heavy metals used increased from 0.5 to 50 ppm. Akinete formation in *A. iyengarii* and zoosporangium formation in *C. glomerata* and *R. hieroglyphicum* were totally suppressed at 5 ppm of any of heavy metals used. However *W. prolifica* and *N. lobatus* vegetative cells could differentiate into akinetes even at 10 ppm of any of heavy metals used. Viability of akinetes or zoosporangia of different algae were affected drastically when formed in presence of any of heavy metals used.
2. *A. iyengarii* and *P. oedogonia* were much sensitive to all heavy metals used than rest of other algae studied.

3. Both the processes of zoosporangium formation and zoospore germination in *C. glomerata* and *R. hieroglyphicum* were comparatively much sensitive to all heavy metals used than were those of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia.*

Effects of Growth hormones on the survivability of vegetative cells, formation of akinetes or zoosporangia, their viability and germination of akinetes or zoospores in different algae used

Methodology

IAA, GA_3 and kinetin were dissolved, separately, in a minimal volume of 80% ethanol and mixed into a known amount of autoclaved and cool culture medium so as to prepare hormones solution of 0.1-500 p.p.m. concentrations. The pH of the medium was adjusted to 7.5. Controls were maintained in basal medium containing the same amount of ethanol as in the particular solution of hormone used. The survivability of vegetative cells and the formation of akinetes and zoosporangia, their viability and the germination of akinetes and zoospores all were determined as usual.

Findings and conclusion

1. The survivability of vegetative cells in all algae, formation of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and the formation of zoosporangia in *C. glomerata* and *R. hieroglyphicum*, all were maximum (that is more than control) at 0.1 ppm of any of growth hormones used. In all algae studied, the survivability of vegetative cells and the formation of reproductive

cells (akinetes or zoosporangia) decreased with an increase in level of growth hormones from 1.0 to 100 ppm. All algae died at 500 ppm of any of all hormones used.

2. Akinetes or zoosporangia formed at lower concentrations (0.1-1.0 ppm) of any of hormones used were similarly viable like those of control akinetes or zoosporangia, but those formed at higher concentrations (10-100 ppm) showed less viability or no viability at all.

3. Akinete or zoospore germination in any of algae studied was maximum at 0.1 ppm of IAA, GA_3 and kinetin. The germination of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia* and zoospores in *C. glomerata* and *R. hieroglyphicum* decreased progressively with an increase in level of growth hormones IAA and kinetin from 10 to 100 ppm. However, GA_3 favoured akinetes and zoospores germination even up to 500 ppm level.

From the present study it is evident that all life cycle stages of various algae studied like survivability of vegetative cells in all algae, the formation and germination of akinetes (as in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia*) or those of zoosporangia and zoospores (as in *C. glomerata* and *R. hieroglyphicum*) all decreased at various levels or inhibited altogether due to any of chemical stresses imposed like shortage or abundance of nutrients, extremes of pH, presence of different pesticides like carbofuran, 2,4-D, dithane, phorate or bavistin or heavy metals like copper sulphate, zinc oxide, mercuric chloride, lead nitrate or

cobalt nitrate. Thus the formation of both akinetes as well as zoosporangia in present algae was not a consequence of any chemical stress imposed but was directly linked with survivability and growth of vegetative cells, since all life cycle stages in different algae studied behaved similarly to any of the chemical stress given. The formation of zoosporangia and germination of zoospores in *C. glomerata* and *R. hieroglyphicum* were comparatively more sensitive processes to any of the chemical stress studied than were those of akinetes in *A. iyengarii, W. prolifica, N. lobatus* and *P. oedogonia.*

Growth hormones IAA, GA_3 and Kinetin at lower concentrations of 0.1-1.0 ppm favoured all life cycle stages of different algae used but at higher concentration of 10-500 ppm suppressed the survivability of vegetative cells and the formation of akinetes or zoosporangia in all algae studied. But GA_3 could promote akinete and zoospore germination even upto 500 ppm level. Actual mechanism of an increase in survivability of algal vegetative cells, or that of formation of reproductive organs following hormonal treatment is not known. Much more work is needed in this area of research.

REFERENCES

REFERENCES

Adhikary, S.P. (1989) : Effect of pesticides on the growth, photosynthetic oxygen evolution and nitrogen fixation of *Westiellopsis prolifica*. *J. Gen. Appl. Microbiol.* **35** : 319-325.

Adhikary, S.P. (1998) : Interaction of Cyanobacteria with Pesticides. *Adv. in Phycology* : 221-250.

Agrawal, S.C. (1984) : Effects of different factors on the akinete germination of the green alga *Stigeoclonium pascheri* (Vischer) Cox and Bold. *Microbios letters.* **27** : 141-144.

Agrawal, S.C. (1985) : Influence of different factors on the zoospore germination and growth of germling in a green alga *Stigeoclonium pascheri* (Vischer) Cox and Bold. *Phykos* **24** : 175-179.

Agrawal, S.C. (1998) : Nutrient induced regulation of reproduction in algae. *Adv. in Phycology*, APC Publications, New Delhi, pp. 187-208.

Agrawal, S.C. & Sarma, Y.S.R.K. (1982a) : Effects of some physical factors and pH on the sporulation of green alga *Stigeoclonium pascheri* (Vischer) Cox and Bold. *Indian J. Bot.* **5** : 151-154.

Agrawal, S.C. & Sarma, Y.S.R.K. (1982b) : Effects of nutrients present in Bold's basal medium on the green alga *Stigeoclonium pascheri*. *Folia Microbiol.* **27** : 131-137.

Agrawal, S.C. & Sarma, Y.S.R.K. (1984) : Effects of indoleacetic acid and gibberellic acid on the spore germination, survival of vegetative colony and sporulation of the green alga *Stigeoclonium pascheri* (Vischer) Cox and Bold. *Adv. Biosc.* **3** : 71-74.

*Ahmad, M.R. & Winter, A. (1969) : *Planta.* **88** : 61.

Ahmad, M.H. and Venkataraman, G.S. (1973) : Tolerance of *Aulosira fertilissima* to pesticides. *Curr. Sci.* **42** : 108-114.

*Altman, H., Fetter, F. & Kaindl, K. (1968) : Untersuchungen Einfluss von Zn-ionen aufclie m-RNA. Synthase in *Chlorella*. 2. Naturf. **23b** : 395-396.

Anderson, R.G. (1960) : The growth and reproduction of *Chara* in a definable nutrient medium. *Diss. Abstr.* **20** (8) : 3034.

Bernstein, E. & John, T.L. (1955) : Certain aspects of two species of *Chlamydomonas*. *J. Protozool.* **2** : 81-85.

Bishop, N.I. (1964) : Site of action of copper in photosynthesis. *Nature* **204** : 401-402.

Capasso, L. & Pinto, G. (1982) : Resistance of the alga *Spermatozopsis acidophila* Kalina (Chlorophyta, Volvocales) to heavy metals. *G. Bot. Ital.* **116** (5-6) : 275-282.

Cembella, A.D., Antia, N.J. & Harrison, P.J. (1984) : The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae. A multidisciplinary perspective. Part I - CRC Critic. *Rev. Microbiol.* **10** : 317-391.

Chapman, A.D. & Pfiester, L.A. (1995). The effects of temperature, irradiance and nitrogen on the encystment and growth of the fresh water dinoflagellates *Peridinium cinctum* and *P. willei* in culture (Dinophyceae). *J. Phycol.* **31** : 355-359.

Chaudhary, B.R. & Singh, H.V. (1986) : Effects of mercuric chloride on akinete germination and sporulation in *Pithophora oedogonia* (Mont.) Wittrock (Cladophorales, Chlorophyceae). *J. Basic. Microbiol.* **26** : 3-8.

Coleman, A.W. (1962) : Sexuality. In : *Physiology and Biochemistry of Algae* Ed. R.A. Lewin Academic Press, New York : 711-729.

Coleman, J.E. (1984) : Carbonic anhydrase : Zn and the mechanism of catalysis in : Biology and chemistry of the carbonic anhydrase. (Eds). Tashian, R.E. and Hewett-Emmett, D. Ann. New York Acad. Sc., New York. **429** : 26-48.

Conrad, H., Saltman, P. and Eppley, R. (1959) : Effects of auxin and gibberellic acid on growth of *Ulothrix*. *Nature* **184** : 556-557.

Constantopoulos, G. (1970) : Lipid metabolism of manganese deficiency on the greening and the lipid composition of *Euglena gracilis*. *Z. Plant Physiol.* **45** : 76-80.

*Davies, A.G. (1976) : An assessment of the basis of mercury tolerance in *Dunaliella tertiolecta*. J. Mar. Biol. Assoc. U.K. 56 : 39-57.

Davis, C.O., Harrison, P.J. & Dugdale, R.C. (1973) : Continuous culture of marine diatom under silicate limitation. I. Synchronized life cycle of *Skeletonema costatum*. *J. Phycology*. **9** : 175-180.

De Filippis, L.F., Pallaghy, C.K. (1994) : Heavy metals : Sources and biological effects. In : *Algae and Water pollution* (Eds) Rai, L.C., Gaur, J.P. and Soeder, C.J., E. Schweizerbartsche Variagsbuchhandlung, Stuttgart : 31-77.

Desikachary, T.V. (1959) : Cyanophyta. Ind. Council of Agric. Res., New Delhi, India.

Dikshit, G. & Tiwari, G.L. (1990) : Effect of pesticide on nitrogen fixing cyanobacteria. Proc. Natl. Symp. Cyanobacterial Nitrogen fixation. I.A.R.I., New Delhi : 495-500.

Driessche, T.V. (1984) : Temporal morphology and cap formation in *Acetabularia*. 2. Effects of morphactin and auxin. *International J. Chronobiology*. **1** : 113-120.

*Dring, M.J. (1967) : Photoperiodic studies on algae. Ph.D. Thesis, University of London.

Dring, M.J. (1974) : Reproduction. In: *Algal physiology and Biochemistry*. Ed. W.D.P. Stewart. Blackwell, Oxford : 814-837.

*Eichenberger, E. (1986) : The interrelation between essentiality and toxicity of metals in the aquatic ecosystem. In : *Metal ions in biological systems* (Eds) Sigel, H. and Sigel, A. Marcel Dekkar Inc., New York and Basel : 67-100.

Erben, K. (1962) : Sporulation In : *Physiology and Biochemistry of algae.* Ed. R.A. Lewin. Academic Press, New York : 701-710.

*Ernst, A. (1908) : Beitrage Zur Morphologie und Physiologie von *Pithophora. Ann. fard. Bot. Buitenzorg.* **7** : 18-55.

Eyster, C., Brown, T.E., Tanner, H.A. and Hood, S.L. (1958) : Manganese requirement with respect to growth, Hill reaction and photosynthesis. *Plant Physiol.* **33** : 235-241.

Fogg, G.E., Stewart, W.D.P., Fay, P. & Walsby, A.E. (1973) : The Blue green algae. Academic Press, London.

Gachter, R., Chou, K.L.S. & Chou, Y.K. (1973). Complexing capacity of the nutrient medium and its relation to inhibition of algal photosynthesis by copper *Schweizerische Zeifschrift fur hydrobiol.* **35** : 252-261.

Gentile, J.H. & Maloney, T.E. (1969) : Toxicity and environmental requirements of a strain of *Aphanizomenon flos-aquae* (L.) Ralf. Canadian, *J. Microbiol.* **15** : 165-173.

Giles, K.L. (1970) : The phytochrome system, phenolic compounds and aplanospore formation in a lichenized strain of *Trebouxia. Canadian J. Bot.* **48** : 1343-1346.

Gregory, W.W., Reed, J.K. and Priester, L.E. (1969) : Accumulation of Parathion and DDT by some algae and protozoa. *J. Protozool.* **16** : 69-71.

Greenfield, S.S. (1942) : Inhibitory effects of inorganic compounds on photosynthesis in chlorella. *Ann. J. Bot.* **29** : 121-131.

Gross, R.E., Punso, P. and Dugger, W.M. (1970) : Observation on the mechanism of copper damage in *Chlorella*. *Plant Physiol.* **46** : 183-185.

Hall, A. (1980) : Heavy metal co-tolerance in a copper tolerant population of marine fouling alga *Ectocarpus siliculosus* (Dillw) Lingbye. *New Phytol.* **85** : 73-78.

Harding, J.P.C. and Whitton, B.A. (1976) : Resistance to zinc of *Stigeoclonium tenue* in the field and the laboratory. *Br. Phycol. J.* **11** : 417-426.

Hirang, T., Shiraishi, K. and Nakano, K. (1955) : Studies on the blue-green algae in low land paddy soil. Part I, on some conditions for the growth of BGA in paddy soil and its effect on growth of paddy rice plant. *Shikoku Nogyo Shikenjo Hokoku* **2** : 121.

Hsiao, S.I.C. & Druehl, L.D. (1973) : Environmental control of gametogenesis in *Laminaria saccharina* II. Correlation of nitrate and phosphate concentrations with gametogenesis and selected metabolities. *Canadian J. Bot.* **51** : 829-839.

Huber, A.L. (1985) : Factors affecting the germination of akinetes of *Nodularia spumigena* (Cyanobacteriaceae). *Appl. Environ. Microbiol.*, **49** : 73-78.

Hughes, E.O.; Gorham, P.R. and Zehuder, A. (1958) : Toxicity of a unialgal culture of *Microcystis aeruginosa*. *Can. J. Microbiol.* **4** : 225-236.

*Hustede, H. (1957) : Untersuchungen uber die stoffliche Beeinflussung der Entwicklung von *Stigeoclonium falklandicum* and *Vaucheria sessilis* durch *Tryptophan abkommlinge. Biol. Zentr.* **76** : 555-556.

*Hustede, H. (1964) : Entwiklungsphysiologische untersuchungen uber den Generations wechsel Zwischen *Derbesia neglecta Berth und Bryopsis halymeniae* Berth. *Bot. Marina.* **6** : 134-412.

Hutchinson, G.E. (1957). A treatise and limnology Vol. I. Geography, physics and Chemistry (Ed. J. Wiley & Sons), p. 1015.

Imahori, K. and Iwasa, K. (1965) : Pure culture and chemical regulation of the growth of charophytes. *Phycologia* **4** : 127-134.

Ishiura, M. (1976) : Gametogenesis of *Chlamydomonas* in the dark. *Plant Cell Physiol.* **17** : 1141-1150.

*Jennings, R.C. (1968) : *Planta.* **80** : 34.

*Jost, D.N. (1953) : *Studies on the vegetative growth and sexual reproduction of Zygnema circumcarinatum* Czurda. Ph.D. Thesis, Harvard University, Cambridge, Massachusetts.

Kar, S. and Singh, P.K. (1979a) : Detoxification of pesticides carbofuran and hexachlorocyclohexane by blue-green algae *Nostoc muscorum* and *Wollea bharadwajae. Microb. Lett.* **10** : 111-114.

Karel-Tetik, N.J. & Sulek, J. (1987) : Sensitivity of the sexual phase of life cycle of *Chlamydomonas geitleri* to the pH changes of media. *Arch. fur. Protistenkunde.* **134** : 115-124.

Katoh, S., Sugi, I., Shiratori, I. and Takamya, I. (1961) : Distribution of plastocyanin in plants, with special reference to its localization in Chloroplasts. *Arch. Biochem. Biophys.* **94** : 136-141.

Kaushik, M., Kumar, H.D. & Singh, H.N. (1971) : Studies on growth and development of two nitrogen fixing blue-green algae. I. Carbon and phosphorus nutrition. *Zietschrift fur Pflanzenphysiologe.* **65** : 432-442.

Kim, W.K. & Greulach, V.A. (1961) : Promotion of algal growth by IAA, GA and Kinetin. *Pl. Physiol.* **36** Supp. XI.

Kimimura, M. and Katoh, S. (1972) : Studies on electron transport associated with photosystem II. Functional site of plastocyanin. Inhibitory effect of $HgCl_2$ on electron transport and plasto cyanin in chloroplast. *Biochim. Biophys. Acta.* **283** : 279-292.

Kojima, Y., Hiyama, T. and Sakurai, H. (1987) : Effect of mercurials on iron sulphur centers of PSI of *Anacystis nidulans.* In *Progress in Photosynthesis Research* (Ed.) Biggins, J., Nijhoff / Junk. The Hague, pp. 57-60.

*Kretschmer, H. (1930) : Beitrage zur cytologie von *Oedogonium Arch. fur. Protistenkunde* **71** : 101-138.

Lang, N.J. (1980) : Akinetes, resistant spores of advanced Cyanobacteria. *J. Phycol. Suppl.* **16** : 24.

Mallick, N. and Rai, L.C. (1990) : Effect of heavy metals on the biology of a nitrogen fixing Cyanobacterium *Anabaena doliolum. Toxicity Assess.* **5** : 207-219.

Martin, T.G. & Whitton, B.A. (1987) : Influence of phosphorus on the morphology and physiology of fresh water *Chaetophora, Draparnaldia* and *Stigeoclonium*, Chaetophorales, Chlorophyta. *Phycologia.* **26** : 59-69.

Mason, C.P. (1965) : Ecology of *Cladophora* in farm ponds. *Ecology* **46** : 421-429.

Megharaj, M., Venkatswarelu, K. and Rao, A.S. (1986) : Effect of monocrotophos and Quinalphos on soil algae. *Environ. Pollut. Ser.* **40** : 121-126.

*Megharaj, M., Venkatswarelu, K. and Rao, A.S. (1988) : Tolerance of algal population in rice soils to carbofuran application. *Curr. Sci.* **57** : 100-102.

Mishra, A.K., Pandey, A.B. and Kumar, H.D. (1989) : Effects of three pesticides on MSX-induced ammonia photo-production by the cyanobacteria *Nostoc linckia. Ecotox. Environ. Saf.* **18** : 145-148.

Moore, R.B. (1967) : Algae as biological indicators of Pesticides. *J. Phycol.* **3** : 4.

Moss, B. (1967) : The culture of fertile tissue of *Fucus vesiculosus. British Phycol. Bull.* **3** : 209-212.

Nasr, A.H. and Bakheet, I.A. (1970) : Effect of certain trace elements and soil extract on some marine algae. *Hydrobiologia.* **36** : 53-60.

O'Kelley, J.C. (1984) : Nitrogen and gamete production in *Chlorococcum echinozygotum* chlorophyceae. *J. Phycol.* **20** : 220-225.

O'Kelley, J.C. & Deason, T.R. (1962) : Effect of nitrogen, sulfur and other factors on zoospore production by *Protosiphon botryoides. Amer. J. Bot.* **49** : 771-777.

Palenic, B. & Morel., F.M.M. (1991) : Amine oxidases of marine phytoplankton. *Appl. Environ. Microbiol.* **57** : 2440-2443.

Pandey, K.D. & Kashyap, A.K. (1987) : Factors affecting formation of spores akinetes in Cyanobacterium *Anabaena doliolum*, Ads. strain. *J. Plant Physiol.* **127** : 123-134.

Pandey, R.K. & Talpasayi, E.R.S. (1980) : Control of sporulation in a blue green alga *Nodularia spumigena* Mertens. *Indian J. Bot.* **3** : 128-133.

Pecora, R.A. & Russell, C.R. (1973) : Zoospore production in selected xanthophycean algae. *British Phycol. J.* **8** : 321-324.

Prescott, G.W. (1962) : Algae of the western great lakes Area. Cranbook Institute of Science, Bloomfield Hills, Michigan.

Price, C.A. (1962) : A zinc-dependent lactate dehydrogenase in *Euglena gracilis. Biochem. J.* **82** : 61-66.

Price, N.M. & Morel, F.M.M. (1994) : Trace metal nutrition and toxicity in Phytoplankton. In *Algae and water pollution* (Eds.). Rai, L.C., Gaur, J.P. and Soeder, C.J., E. Schweizerbartsche Varlagsbuch hundlung, Stuttgart. pp. 79-91.

Provasoli, L. (1958) : Effect of plant hormones on *Ulva. Biol. Bull.* **114** : 375.

Rai, A.K. & Pandey, G.P. (1981) : Influence of environmental stress on the germination of *Anabaena vaginicola* akinetes. *Ann. Bot.* **48** : 361-370.

Rai, L.C. & Dubey, S.K. (1989) : Impact of chromium and tin on a nitrogen fixing cyanobacterium *Anabaena doliolum.* Interaction with bivalent cations toxicol. *Exotoxicol. Environ. Safety.* **17** : 94-104.

Rai, L.C., Gaur, J.P. & Kumar, H.D. (1981a) : Phycology and heavy metal pollution. *Biol. Rev.* **56** : 99-151.

Rai, L.C. & Raizada, M. (1986) : Nickel induced stimulation of growth, heterocyst differentiation, $^{14}CO_2$ uptake and nitrogenase activity in *Nostoc muscorum. New Phytol.* **104** : 111-114.

Rai, L.C. and Raizada, M. (1989) : Effect of bimetallic combination of Ni, Cr and Pb on growth, uptake of nitrate and ammonia, $^{14}CO_2$ fixation and nitrogenase activity of *Nostoc muscorum. Ecotoxicol. Environ. Safety.* **17** : 75-85.

Rai, L.C., M. Raizada, N. Mallick, Y. Husaini, A.K. Singh & S.K. Dubey (1990) : Effect of four heavy metals on the biology of *Nostoc muscorum. Biol. Metals.* **2** : 229-234.

Rai, L.C., Singh, A.K. & Mallick, N. (1991) : Studies on photosynthesis, the associated electron transport system and some physiological variables in *Chlorella vulgaris* under heavy metal stress. *J. Plant. Physiol.* **137** : 419-424.

Rai, A.K. & Pandey, G.P. (1981) : Influence of environmental stress on the germination of *Anabaena vaginicola* akinetes. *Ann. Bot.* **48** : 361-370.

Rai, L.C. & Dubey, S.K. (1989) : Impact of chromium and tin on a nitrogen fixing cyanobacterium *Anabaena doliolum*. Interaction with bivalent cations toxicol. *Exotoxicol. Environ. Safety.* **17** : 94-104.

Rai, L.C., Gaur, J.P. & Kumar, H.D. (1981a) : Phycology and heavy metal pollution. *Biol. Rev.* **56** : 99-151.

Rai, L.C. & Raizada, M. (1986) : Nickel induced stimulation of growth, heterocyst differentiation, $^{14}CO_2$ uptake and nitrogenase activity in *Nostoc muscorum. New Phytol.* **104** : 111-114.

Rai, L.C. and Raizada, M. (1989) : Effect of bimetallic combination of Ni, Cr and Pb on growth, uptake of nitrate and ammonia, $^{14}CO_2$ fixation and nitrogenase activity of *Nostoc muscorum. Ecotoxicol. Environ. Safety.* **17** : 75-85.

Rai, L.C., M. Raizada, N. Mallick, Y. Husaini, A.K. Singh & S.K. Dubey (1990) : Effect of four heavy metals on the biology of *Nostoc muscorum. Biol. Metals.* **2** : 229-234.

Rai, L.C., Singh, A.K. & Mallick, N. (1991) : Studies on photosynthesis, the associated electron transport system and some physiological variables in *Chlorella vulgaris* under heavy metal stress. *J. Plant. Physiol.* **137** : 419-424.

Rao, V.V., Ghosh, R. & Singh, H.N. (1987) : Diazotrophic regulation of akinetes development in the Cyanobacterium *Anabaena doliolum*. *New Phytol.* **106** : 161-168.

Rath, B. and Adhikary, S.P. (1994) : Relative tolerance of several nitrogen fixing cyanobacteria to commercial grade furadan (Carbofuran, 3%). *Ind. J. Expt. Biol.* **32** : 213-215.

Rath, B. and Adhikary, S.P. (1995) : Growth response of two species of *Anabaena* to commercial and analytical grade Furadan. Proc. Internatl. Sympos. In "Impact of Modern Agriculture on Environment". Soc. Sust. Agri. and Max Muller Bhawan, New Delhi. pp. 151-155.

Rayburn, W.R. (1974) : Sexual reproduction in *Pandorina unicocca*. *J. Phycol.* **10** : 258-265.

Reddy, P.M. (1976) : Physiological and biochemical studies on cellular differentiation with special reference to perennating structures of blue green algae. Ph.d. Thesis, Banaras Hindu University, Varanasi.

Rosko, J.J. and Rachlin, J.W. (1977) : The effect of cadmium, copper, mercury, zinc and lead on cell division, growth and chlorophyll a content of the chlorophyte. *Chlorella vulgaris*. *Bull. Torr. Bot. Club.* **104** : 226-233.

*Rowan, M. (1937) : Some responses of *Hydrodictyon reticulatum* to stimulation of germination. Ph.D. Thesis, Columbia University.

Rueter, J.G. and Morel, F.M.M. (1981) : The interaction between zinc deficiency and copper toxicity as it affects the silicic acid uptake mechanism in *Thalassiosira pseudonana. Limnol Oceanogr.* **26** : 67-73.

Saona, S. (1964) : Effect of gibberellic acid on the growth and multiplication of some soil-microorganisms and unicellular green algae. *Nature* **204** : 1328-1329.

Sardeshpande, J.S. and Goyal, S.K. (1982) : Effect of insecticides on the growth and nitrogen fixation by blue-green algae. In : Proceeding of Natl. Symp. Biol. N. Fixation. IARI, New Delhi : 588-605.

Sarma, Y.S.R.K. and Tripathi, S.N. (1974) : Effects of gibberellic acid on green alga, *Oedogonium acmandrium* Elfving. *Ind. J. Exp. Biol.* **12** : 204-206.

Sharma, V.K. (1986) : A review of recent work on pesticide studies on the nitrogen fixing algae. *J. Environ. Biol.* **7** : 171-175.

Sinclair, C. and Whitton, B.A. (1977) : Influence of nutrient deficiency on hair formation in the Rivulariaceae. *British Phycol. J.* **12** : 296-313.

Singh, H.V. and Chaudhary, B.R. (1988) : Effect of Indole acetic acid and gibberellic acid on oogonium formation in *Oedogonium Hatei.* Kam. *Phykos.* **27** : 135-139.

Singh, P.K. (1974): Algicidal effect of 2,4-dichlorophenoxy acetic acid on blue-green alga *Cylindrospermum sp. Arch. Microbiol.* **97** : 69-72.

Spencer, T. (1968) : Effect of kinetin on the phosphatase enzymes of *Acetabularia*. *Nature*. **217** : 62-64.

Stanier, R.Y., Kunisawa, R. Mandel, M., Cohen, B. (1971) : Purification and properties of a unicellular blue-green alga (Order Chroococcales). *Bact. Rev.* **35** : 171-205.

Steele, R.L. (1965) : Induction of sexuality in two centric diatoms. *Bioscience*. **15** : 298.

Stokes, P.M. (1975) : Adaptation of green algae to high levels of copper and nickel in aquatic environments. In : *Proceedings of International Conference of Heavy metals in the Environment.* (Ed.) Hutchinsm, T.C. Toronto, Canada. pp. 137-154.

Stokes, P.M. (1983) : Response of fresh water algae to metals. In *Progress in Phycological Research*, Vol. 2 (F.E. Round, V.J. Chapman, Eds) Elsevier Science Publisher. pp. 87-111.

Subramanian, G., Sekar, S. and Sampoornam, S. (1994) : Biodegradation and utilization of organophosphorus pesticides by cyanobacteria. *Int. Biodet. Biodeg.* **33** : 129-143.

Sundaralingam, V.S. and Govindraj, A.V. (1977) : Effect of IAA and kinetin on the growth of *Cosmarium subtriordinatum* West and West. *Phykos.* **16** : 55-58.

Tanner, H.A., Brown, T.E., Eyster, C. and Trecharne, R.W. (1960) : A manganese dependent photosynthetic process. *Biochem. Biophys. Res. Commun.* **3** : 205-210.

Tanoue, E. and Aruga, Y. (1975) : Studies on the life cycle and growth of *Platymonas* sp. in culture. *Japanese J. Bot.* **20** : 439-460.

Thomas, W.H. & Seibert, D.L.R. (1977) : Effect of copper on the dominance and diversity of algae : Controlled ecosystem pollution experiment. *Bull. Mar. Sci.* **27** : 23-33.

Tiftickjian, J.D. & Rayburn, W.R. (1986) : Nutritional requirements for sexual reproduction in *Mesotaenium kramstai*, Chlorophyta. *J. Phycol.* **22** : 1-8.

Tomson, A.M., Demets, R., Sigon, C.A.M., Stegwee, D. & Van den Ende, H. (1986) : Cellular interactions during the mating response in *Chlamydomonas eugametos. Protoplasma.* **155** : 200-209.

Trainor, F.R. (1959) : A comparative study of sexual reproduction in four species of chlamydomonas. *Amer. J. Bot.* **46** : 65-70.

Van Assche, F. and Clijsters, H. (1990) : Effects of metals on enzyme activity in plants. *Plant Cell Environ.* **13** : 195-206.

Van Den Ende. H., Van Den Briel, M.L., Lingeman, R., Van Der Gulik, P. & Munnik, T. (1992) : Zygote formation in homothallic green alga *Chlamydomonas monoica* Strehlow. *Planta.* **188** : 551-558.

Van Dok, W. & Hart, B.T. (1996) : Akinete differentiation in *Anabaena circinalis* (Cyanophyta). *J. Phycol.* **32** : 557-565.

Van Dok, W. & Hart, B.T. (1997) : Akinete germination in *Anabaena circinalis* (Cyanophyta). *J. Phycol.* **33** : 12-17.

Venkataraman, G.S. (1972) : Algal biofertilizer and rice cultivation. Pub. Today and Tomorrows, New Delhi.

Venkataraman, G.S. & Rajyalakshmi, B. (1972) : Relative tolerance of nitrogen fixing blue green algae to pesticides. *Ind. J. Agric. Sci.* **42** : 119-121.

Vidyavati & Nizam, J. (1973) : Conjugation studies in *Closterium acerosum* Ehren. *Phykos.* **12** : 61-71.

Visviki, I. & Rachlin, J.W. (1992) : Ultrastructural changes in Dunaliella minuta following acute and chronic exposure to copper and cadmium. *Arch. Environ. Contam. Toxicol.* **23** : 420-425.

Von Stosch, H.A. & Fecher, K. (1979) : "Internal thecae" of *Eunotia soleirolii*, Bacillariophyceae. Development, structure and function as resting spores. *J. Phycol.* **15** : 233-243.

*Whitton, B.A. (1973) : Fresh water plankton. In N.G. Carr and B.A. Whitton, eds. The biology of blue green algae. University of California Press.

*Whitton, B.A. (1984) : Algae as monitor of heavy metals in freshwater *Algae as Ecological indicators* (Ed.) Shubert, L.E. Academic Press, London, pp. 257-280.

Wiese, L. & Jones, R.F. (1963) : Studies on gamete copulation in heterothallic chlamydomonads. *J. Cellular Comparative Physiol.* **61** : 265-274.

Wolk, C.P. (1965) : Control of sporulation in a blue green alga. *Dev. Biol.* **12** : 15-35.

Wyman, M. & Fay, P. (1986) : Interaction between light quality and nitrogen availability in the differentiation of akinetes in the planktonic cyanobacterium *Gloeotrichia echinulata. British Phycol. J.* **21** : 147-153.

Xylander, M. & Braune, W. (1994) : Influence of nickel on the green alga Haematococcus lacustris Rostafinski in phases of its life cycle. *J. Plant Physiol.* **144** : 86-93.

Yamamoto, Y. (1976) : Effects of some physical and chemical factors on the germination of akinetes of *Anabaena cylindrica. J. Gen. Appl. Microbiol.* **22** : 311-323.

Yin, H.C. (1937) : Effect of auxin on *Chlorella vulgaris. Proc. Natl. Acad. Sci.* (Wash.) **23** : 174.

---

* Original not seen.